

REVIEW

Open Access



Genomic insights into head and neck cancer

Tim N. Beck^{1,2*} and Erica A. Golemis^{1,2*}

Abstract

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide and is frequently impervious to curative treatment efforts. Similar to other cancers associated with prolonged exposure to carcinogens, HNSCCs often have a high burden of mutations, contributing to substantial inter- and intra-tumor heterogeneity. The heterogeneity of this malignancy is further increased by the rising rate of human papillomavirus (HPV)-associated (HPV+) HNSCC, which defines an etiological subtype significantly different from the more common tobacco and alcohol associated HPV-negative (HPV-) HNSCC. Since 2011, application of large scale genome sequencing projects by The Cancer Genome Atlas (TCGA) network and other groups have established extensive datasets to characterize HPV- and HPV+ HNSCC, providing a foundation for advanced molecular diagnoses, identification of potential biomarkers, and therapeutic insights. Some genomic lesions are now appreciated as widely dispersed. For example, HPV- HNSCC characteristically inactivates the cell cycle suppressors TP53 (p53) and CDKN2A (p16), and often amplifies CCND1 (cyclin D), which phosphorylates RB1 to promote cell cycle progression from G1 to S. By contrast, HPV+ HNSCC expresses viral oncogenes E6 and E7, which inhibit TP53 and RB1, and activates the cell cycle regulator E2F1. Frequent activating mutations in PIK3CA and inactivating mutations in NOTCH1 are seen in both subtypes of HNSCC, emphasizing the importance of these pathways. Studies of large patient cohorts have also begun to identify less common genetic alterations, predominantly found in HPV- tumors, which suggest new mechanisms relevant to disease pathogenesis. Targets of these alterations including AJUBA and FAT1, both involved in the regulation of NOTCH/CTNBN1 signaling. Genes involved in oxidative stress, particularly CUL3, KEAP1 and NFE2L2, strongly associated with smoking, have also been identified, and are less well understood mechanistically. Application of sophisticated data-mining approaches, integrating genomic information with profiles of tumor methylation and gene expression, have helped to further yield insights, and in some cases suggest additional approaches to stratify patients for clinical treatment. We here discuss some recent insights built on TCGA and other genomic foundations.

Keywords: Head and neck cancer, TCGA, HPV, Genomics, Cancer therapy, Cell cycle, Personalized medicine, Tumor heterogeneity

Background

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer, with annual incidence of 600,000 cases worldwide [1]. Anatomically, head and neck cancer regions include the oral cavity, the pharynx (nasopharynx—behind the nose; oropharynx—soft palate, base of the tongue and the tonsils; hypopharynx—the lowest part of the pharynx), the larynx, the paranasal sinuses, the nasal cavity and the salivary glands

[2]. Beyond distinction by anatomic sites, HNSCC is divided into two broad classes: human papillomavirus (HPV)-associated (HPV+) and HPV-negative (HPV-) disease. The majority of HPV-negative HNSCC arises from the larynx and oral cavity [3, 4], although a small fraction of cases originates in the oro- and hypopharynx. HPV+ disease is typically found in the oropharynx, with a minority of cases detected in the larynx and oral cavity [5]. As of 2016, the majority of HNSCC is HPV- disease, and is most commonly associated with tobacco use and heavy alcohol consumption [6]. The exception is oropharyngeal HNSCC, 60-70 % of which is HPV+ in North America and Europe (significant geographic variation

* Correspondence: Tim.Beck@Fccc.edu; Erica.Golemis@fccc.edu

¹Program in Molecular Therapeutics, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111, USA

Full list of author information is available at the end of the article

exists in the prevalence of HPV+ disease worldwide [3, 5, 7]). Over 150 types of HPV have been identified, with HPV subtype 16 (HPV-16) identified as the most oncogenic, detected in over 90 % of HPV+ oropharyngeal cancers [8]. HPV+ HNSCC is typically diagnosed in a younger patient population (6th decade of life; [5, 9]) and its prevalence has dramatically increased since the 1980's (then only detected in 16 % of oropharyngeal cancer; [7, 9]). HPV- HNSCC is generally diagnosed in an older patient population (7th decade of life), often presents with locally advanced or metastatic features, and has a relatively poor prognosis compared to HPV+ tumors [5, 10].

Both HPV+ and HPV- HNSCC are treated with a combination of surgery, radiation and adjuvant chemotherapy. Treatment specifics vary depending on anatomic site and disease stage. In general, low stage tumors are treated with surgery, followed by radiation if positive surgery margins are detected. For more advanced cases treatment includes surgery, if possible, followed by radiation with or without adjuvant chemotherapy [1, 9, 11]. In spite of significant improvements, including the introduction of targeted and immunotherapies (most prominently, immune checkpoint inhibitors targeting cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) [12]), as of 2015 the relative 5-year survival rate is only approximately 25–40 % for HPV- and 70–80 % for HPV+ HNSCC [1, 13, 14]. To fully capture the diversity of HNSCC and to gain clinically meaningful insights that can improve treatment, it seems critical to define the full spectrum of molecular alterations and the heterogeneity associated with this pathology.

At no prior point in time has it been possible to describe the molecular landscape of the various, mostly anatomically defined cancers with as much detail and precision as is possible today [15–17], based on concerted efforts to uncover the genomic (most advanced), epigenomic, proteomic and transcriptomic changes that occur as healthy tissue turns malignant, metastatic and resistant to treatment [18]. The Cancer Genome Atlas (TCGA) network and others have periodically published datasets on many cancers [15, 17], including extensive analyses of HPV- and to a lesser degree HPV+ HNSCC (Table 1; [17, 19–24]). Amongst non-lung and non-skin tumor types, head and neck cancer has one of the highest rates of non-synonymous mutations and a high degree of genomic instability [15, 16, 25, 26], which contribute to the enormous heterogeneity of HNSCC [19, 24]. Since large-scale datasets began to appear in 2011 [20, 22], a number of groups have performed integrated bioinformatics, translational, and clinical analyses that leverage the genomic resources, suggesting new research directions. This review summarizes and highlights

potential therapeutic opportunities in HPV- and HPV+ HNSCC based on the analysis of high throughput data published by the TCGA network and others.

Foundational genomic datasets

The pathophysiological differences between HPV+ and HPV- HNSCC necessitate that genomic analyses apply rigorous classification methods for HPV dependence in clinical samples [10, 22, 27]. HPV status is most commonly determined by polymerase chain reaction (PCR) or *in situ* hybridization (*ISH*) to detect HPV genetic material, or by immunohistochemical (IHC) staining for the tumor suppressor p16 (CDKN2A), which is induced as a consequence of HPV-associated transformation [28]. p16 IHC staining is greatly increased as a result of HPV infection, and is a reliable proxy for positive HPV status in primary tumors of the oropharynx [28]. Virally encoded proteins target the cell cycle regulator retinoblastoma 1 gene (RB1), providing one potential feedback mechanisms for enhancing expression of p16 ([29], and discussed further below). Alternatively, it has been shown that upregulation of p16 can also occur as a cellular response to the infection itself, through induction of the histone 3 lysine-27 (H3K27) specific demethylases KDM6A and KDM6B [30–32]. For other anatomic sites, the true positive rate for p16 IHC staining falls below 50 %, reflecting the rarity of HPV-associated tumors outside of the oropharynx [28, 33]. High p16 expression also occurs in about 5 % of HPV- cases, for reasons that are at present unclear [28]. For these reasons, the TCGA network took extensive measures to ensure proper HPV classification of each tumor: in addition to p16 staining and *ISH*, whole HPV genome sequencing as well as HPV RNA-Seq was performed. HPV positive cases were classified as such if > 1000 RNA-Seq reads aligned to viral genes E6 and E7 [19].

The TCGA network analyzed 243 HPV-negative and 36 HPV-positive tumors using multiple platforms (RNA sequencing, DNA sequencing, reverse phase protein array (RPPA), DNA methylation profiling and miRNA sequencing) to define the molecular landscape of this malignancy [19, 34]. Most of the patients in the TCGA cohort were male (~70 %) and heavy smokers (51 mean pack years; [19]), closely resembling the general HPV- HNSCC patient population [1, 11]. Tumors predominantly originated from the oral cavity ($n = 172$; 62 %; 160/172 HPV- and 12/172 HPV+) and the larynx ($n = 72$; 26 %; 71/72 HPV- and 1/72 HPV+), with only a few cases originating from the oropharynx ($n = 33$; 12 %; 11/33 HPV- and 22/33 HPV+) and only two from the hypopharynx (1/2 HPV+ and 1/2 HPV-).

Beyond the work of the TCGA network, additional genomic sequencing studies (Table 1) were performed by Stransky et al. (53 HPV- and 11 HPV+; [22]), Agrawal

Table 1 High-throughput genomic studies of HNSCC. The most frequently altered genes described in seven studies are shown, separated by HPV status when possible

TCGA (2015; [19]) ^a	Seiwert et al. (2015; [21])	Lin et al. (2014; [37]) ^a	Pickering et al. (2014; [36]) ^b	Pickering et al. (2013; [35])	Stransky et al. (2011; [22]) ^a	Agrawal et al. (2011; [20]) ^a
HPV- <i>n</i> = 243	HPV- <i>n</i> = 69	N/A (NPC) <i>n</i> = 128	N/A (Tongue) <i>n</i> = 34 (YT 16, OT 28)	N/A (OSCC) <i>n</i> = 35-40	HPV- <i>n</i> = 63	N/A <i>n</i> = 28
TP53 (84 %, M)	TP53 (81 %, M)	TP53 (17 %, M/D)	TP53 (94 %, 57 %, M)	CDKN2A (74 %, D)	TP53 (73 %, M)	TP53 (79 %, M)
CDKN2A (57 %, M/D)	CDKN2A (33, M/D)	CDKN2A/B (13 %, M/D)	CSMD1 (25 %, 75 %, D)	TP53 (66 %, M)	CDKN2A (25 %, M/D ^c)	NOTCH1 (14 %, M)
let-7c (40 %, miRNA)	MDM2 (16 %, A)	ARID1A (11 %, M/D)	PIK3CA (0 %, 11 %, M); (30 %, 70 %, A)	FAT1 (46 %, M/D)	SYNE1 (22 %, M)	RELN (14 %, M)
PIK3CA (34 %, M/A)	MLL2 (16 %, M)	SYNE1 (8 %, M)	CDKN2A (6 %, 4 %, M); (55 %, 65 %, D)	TP63 (26 %, A)	CCND1 (22 %, A ^c)	SYNE1 (14 %, M)
FADD (32 %, A)	NOTCH 1 (16 %, M)	ATG13 (6 %, M/D)	FADD/CCND1 (40 %, 65 %, A)	CCND1 (23 %, A)	MUC16 (19 %, M)	EPHA7 (11 %, M)
FAT1 (32 %, M/D)	CCND1 (13 %, A)	MLL2 (6 %, M)	FAT1 (6 %, 25 %, M); (50 %, 35 %, D)	MAML1 (23 %, D)	USH2A (18 %, M)	FLG (11 %, M)
CCND1 (31 %, A)	PIK3CA (13 %, M)	PIK3CA (6 %, M/A)	EGFR (20 %, 50 %, A)	EGFR (17 %, A)	FAT1 (14 %, M)	HRAS (11 %, M)
NOTCH1/2/3 (29 %, M/D)	PIK3CB (13 %, M/A)	CCND1 (4 %, A)	NOTCH1 (25 %, 18 %, M)	TNK2 (17 %, A)	LRP1B (14 %, M)	PIK3AP1 (11 %, M)
TP63 (19 %, A)	UBR5 (13 %, M/D)	NOTCH3 (4 %, M)	HLA-A (0 %, 14 %, M)	AKT1 (14 %, A)	ZFH4 (14 %, M)	RIMBP2 (11 %, M)
EGFR (15 %, M/A)	EGFR (12 %, A)	FGFR2 (4 %, M)	CASP8 (6 %, 11 %, M)	SRC (14 %, A)	NOTCH1 (13 %, M)	SI (11 %, M)
HPV+ <i>n</i> = 36	HPV+ <i>n</i> = 51				HPV+ <i>n</i> = 11	HPV+ <i>n</i> = 4
E6/7 (100 %)	E6/7 (100 %)				E6/E7 (100 %)	E6/E7 (100 %)
PIK3CA (56 %, M/A)	PIK3CA (22 %, M)				PIK3CA (27 %, M)	EPHB3 (25 %, M)
TP63 (28 %, A)	TP63 (16 %, M/A)				RUFY1 (18 %, M)	UNC5D (25 %, M)
TRAF3 (22 %, M/D)	PIK3CB (13 %, M/A)				EZH2 (18 %, M)	NLRP12 (25 %, M)
E2F1 (19 %, A)	FGFR3 (14 %, M)				CDH10 (18 %, M)	PIK3CA (25 %, M)
let-7c (17 %, miRNA)	NF1/2 (12 %, M)				THSD7A (18 %, M)	TM7SF3 (25 %, M)
NOTCH1/3 (17 %, M)	SOX2 (12 %, A)				FAT4 (18 %, M)	ENPP1 (25 %, M)
FGFR3 (11 %, F/M)	ATM (10 %, D)				KMT2D (18 %, M)	NRXN3 (25 %, M)
HLA-A/B (11 %, M/D)	FLG (12 %, M)				ZNF676 (18 %, M)	MICAL2 (25 %, M)
EGFR (6 %, M)	MLL3 (10 %, M)				MUC16 (18 %, M)	

N/A HPV status not available, NPC nasopharyngeal cancer, YT young tongue, OT old tongue, OSCC oral squamous cell carcinoma, M mutation, A amplification, D deletion, F fusion

^aData was accessed using cBioportal [38, 39]

^bvalues for A and D are approximations

^cpercentages are not based on the 63 cases, because CNAs were not analyzed for all cases

et al. (28 HPV- and 4 HPV+; [20]), Pickering et al. (40 oral squamous cell carcinoma; likely HPV-negative; [35]), Seiwert et al. (69 HPV- and 51 HPV+; [21]), and Pickering et al. (34 squamous cell carcinoma of the oral tongue; likely HPV-negative; [36]). These predominantly relied on a single platform (exome/massively parallel sequencing) for data acquisition. In addition, Lin et al. have sequenced 128 cases of nasopharyngeal carcinomas (NPC; likely HPV-; [37]).

The TCGA dataset [19] is conveniently accessible through cBioportal [38, 39], as are the datasets from Stransky et al., Agrawal et al. [20, 22], and the NPC study [37]. The 279 patient TCGA dataset provides the most extensive tumor profiles, including mutational data from whole exome sequencing, identification of somatic copy number alterations using the GISTIC algorithm [40], mRNA expression data (RNA-Seq V2 RSEM), and protein expression data for a total of 165 combined

phosphoproteins and proteins (reverse-phase protein array/microarray; [38, 39, 41]). The other three datasets accessible through cBioPortal predominantly cover somatic mutations. The dataset for Pickering et al. [35] is available through Gene Expression Omnibus (GEO; [42]); the remaining studies provide access to datasets via links provided in the original publications. Table 1 summarizes the top alterations detected in each study for HPV- and, if available, HPV+ cases. Certain alterations were detected across several studies; whereas, a significant number of alterations were not uniformly detected, potentially due to the variation in detection platforms, disease heterogeneity and significant demographic differences.

Common genomic defects: HPV+ and HPV-

Copy number alterations (CNA) are frequent in HNSCC [26] and are highly concordant across most of the genome for HPV- and HPV+ cases [19, 43]. One of the most frequently amplified regions (in approximately 15–30 % of cases [19]) is on chromosome 3q and includes the anti-apoptotic kinase protein kinase C (PIK3CA),

and the transcription factors TP63 and SOX2 [44]. Additional amplifications found in both the HPV+ and HPV- disease subtypes include chromosomes 5p and 8q [19, 43], which encompass telomerase TERT (5p) and the oncogene MYC (8q). Commonly seen deletions prominently cover parts of chromosome 3p and 8p, impacting two tumor suppressor genes: FHIT (3p; expression loss is associated with worse survival in HNSCC [45]) and CSMD1 (8p; [19, 46, 47]). Losses in 3p and 8p and gains in 3q, 5p and 8q are also frequently seen in squamous cell carcinomas (SQCC) of the lung [48], highlighting important genomic similarities between SQCC and HNSCC [19, 43, 48].

The microRNA let-7c, a cell cycle regulator, is frequently inactivated in both HPV- and HPV+ HNSCC in the TCGA cohort (Fig. 1a and b). Depressed expression of let-7-c is associated with increased expression of CDK4, CDK6, E2F1 and PLK1, kinases and translational regulators important for progression through the cell cycle [19, 49]. In depth analysis of TCGA microRNA data has been used to test the hypothesis that expression of 28 microRNAs selected based on *in vitro* experiments

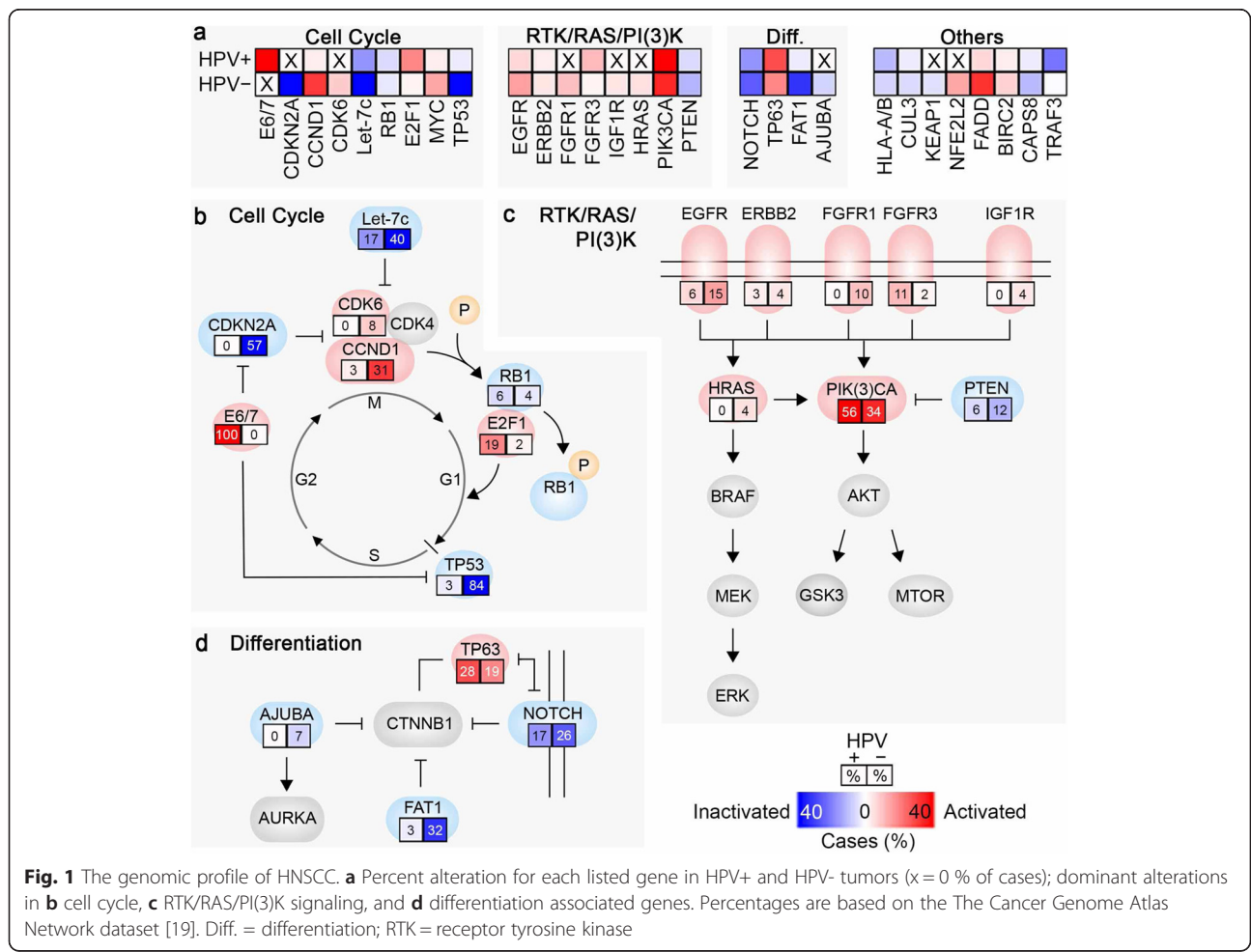


Fig. 1 The genomic profile of HNSCC. **a** Percent alteration for each listed gene in HPV+ and HPV- tumors (x = 0 % of cases); dominant alterations in **b** cell cycle, **c** RTK/RAS/PI(3)K signaling, and **d** differentiation associated genes. Percentages are based on the The Cancer Genome Atlas Network dataset [19]. Diff. = differentiation; RTK = receptor tyrosine kinase

could predict response to radiotherapy [50]. Patients from the TCGA cohort with complete clinical annotations were divided into three groups: radiation with complete response (radiosensitive), radiation with tumor progression (radioresistant), and not irradiated. This analysis suggested that upregulation of miR-016, miR-29, miR-150, miR-1254 and downregulation of let-7e correlated with complete response to radiotherapy. Effects were linked to ATM expression. Higher levels of ATM correlated with increased radio-resistance, based on RPPA data also provided by the TCGA [50]. These interesting findings necessitate validation using additional cohorts, but clearly indicate the potential value of analyzing microRNA expression in HNSCC.

One of the most commonly activated (mutationally or due to amplification of the 3q chromosomal region) genes in both HPV+ and HPV- cases in the TCGA cohorts (56 and 34 %, respectively) and other studies (Table 1; [21–23]) is PIK3CA, encoding the p110 α catalytic subunit of phosphoinositol-3-kinase (Fig. 1; [19]). In this regard, HNSCC is similar to many other cancers in which PIK3CA is amongst the most commonly mutated genes [18]. PIK3CA encodes a lipid kinase that regulates signal propagation from multiple input sources [51], including many of the receptor tyrosine kinases (RTKs) relevant to HNSCC (Fig. 1c; [19, 52, 53]). Functionally, PIK3CA regulates phosphorylation of AKT1, and mutated PIK3CA has been shown to attenuate apoptotic signals and support tumor invasion [54]. Additionally, mutationally activated PIK3CA has been shown to support cyclin D activity [55]; thus, further emphasizing the tremendous relevance of cell cycle dysregulation in head and neck cancers [43, 56].

Lui et al. performed a focused whole-exome analysis of 151 HNSCC tumors (datasets from Stransky et al. and Agrawal et al., plus 45 additional cases [20, 22]), specifically exploring PI3K pathway mutations for therapeutic opportunities [23]. This analysis (which did not assess CNAs) indicated the PI3K pathway was the most frequently mutated oncogenic pathway (30.5 % of tumors, 46/151 [23]). PIK3CA in particular was mutated in 12.6 % of cases (19/151; [23]), which is substantially less than the number of cases with mutated PIK3CA (21 %, 58/279) reported by the TCGA [19]. Nevertheless, assessment of patient derived xenografts expressing wild type PI3KCA or mutant PI3KCA and treated with vehicle or the mTOR/PI3K inhibitor BEZ-235 [57] indicated tumors with mutated PI3KCA were exquisitely sensitive to the small molecule inhibitor, whereas tumors without the mutation did not respond to the treatment [23]. Several studies using pre-clinical models also demonstrated that HNSCC with wild type PI3KCA is sensitive to PI3K/mTOR inhibitors, particularly in combination with a MEK inhibitor or in combination with radiation in the

context of wild type p53 [58, 59]. Development of a large number of PI(3)K inhibitors is ongoing, with several promising compounds currently being tested in clinical trials [60].

Another commonly altered gene in HNSCC is NOTCH1. NOTCH1 is a transmembrane kinase frequently mutationally inactivated (most commonly via missense or truncating mutation) in both HPV+ and HPV- cases (13–26 % and 8–17 %, respectively [19, 21]; Table 1). The role of the NOTCH pathway is complicated and depends on the overall organization of the broader signaling network and on the specific tissue type [61]. Exome sequencing of HNSCC strongly implicated NOTCH1 as a tumor suppressor in this malignancy, as close to 40 % (11/28) of NOTCH1 mutations were truncating mutations predicted to be inactivating [20]. This conclusion was supported by the observation that NOTCH1 knockout mice developed tumors due to increased oncogenic CTNNB1 signaling [62]. Additional work in tongue carcinoma cells observed robust downregulation of CTNNB1 in the background of stable expression of NOTCH1 [63]. Another important feature of NOTCH1 is its participation in reciprocal negative regulation with p63 [64], a member of the p53 family found to be activated with high frequency in HNSCC (19 and 28 % in HPV- and HPV+ cases respectively, mostly due to amplification [19]). In keratinocytes, overexpression of p63 induced cell growth in part by suppression of p21 and thus directly counteracting the growth suppressive input from NOTCH1 [65]. The TCGA data supports this NOTCH1-p63 paradigm in HNSCC, given the high incident of NOTCH1 inactivating mutations and the significant incident of p63 activation. Of note, p63 is transcriptionally activated by two distinct promoters [66]; one of the two resulting p63 variants contains an N-terminal transactivation domain (TAp63), whereas, the other transcript lacks the N-terminal domain and is termed Δ Np63 [66]. The two p63 isoforms are functionally distinct [67], with Δ Np63 acting as a dominant negative regulator of p53 and with TAp63 opposing cell cycle arrest and apoptosis [66, 68]. Δ Np63 is highly expressed in HNSCC [69] and indeed inhibits NOTCH1 activity [65, 70].

HPV-Negative HNSCC

Well prior to the advent of high throughput sequencing, alterations in several genes, including inactivation or deletion of the tumor suppressors CDKN2A (p16; [71]) and TP53 (p53; [72]), and overexpression (via amplification and elevated transcription) of the epidermal growth factor receptor EGFR [73] had been identified as relevant to the pathogenesis of HPV-negative HNSCC. Based on TCGA analysis of genomic-scale data, the two most commonly inactivated genes in HPV- tumors were confirmed as TP53 (84 % of cases [19]; a percentage

similar to the one reported by Seiwert et al. (81 % of 69 HPV- tumors; [21]) and Stransky et al. (73 % of 63 HPV- tumors; [22]) and CDKN2A (4–74 % of cases Fig. 1a and b and Table 1; [19, 21, 22]; the broad range is in part due to the lack of CNA data for several of the studies and the difficulties associated with sequencing of GC-rich regions, which are found in CDKN2A [74, 75]).

Due to the high frequency of mutations in TP53 (Table 1), significant effort has been focused on elucidating the prognostic potential of this gene. Some work suggests improved overall survival for patients with wild type TP53 compared to patients with TP53 mutations predicted to be functionally disruptive (i.e., nonsense mutations or missense mutations disruptive to the L2 or L3 DNA-binding domains [76]). Other reports indicated that TP53 status is of low prognostic value when considered independently from other variables [77]. A multi-tiered genomic analysis of 250 HPV-negative tumors in 2014 (TCGA dataset; approximately corresponding to the cohort described above) confirmed that disruptive TP53 mutations correlated with reduced survival; however, in this analysis, cases with TP53 mutations predicted to be non-disruptive also had significantly worse survival outcomes compared to cases with wild type TP53 [78]. Strikingly, this study identified TP53 mutations as frequently co-occurring with deletions of chromosomal region 3p (179 out of 250 cases), with the combination associated with significantly worse survival than was predicted for TP53 mutations or 3p deletions considered independently [78]. Further stratification of the 179 TP53-3p cases showed that elevated expression of miR-548k (a microRNA encoded by a gene proximal to cyclin D1 (CCND1) and the death receptor FADD at 11q13 and described as oncogenic in esophageal squamous cell cancer [79]) predicted further reduction in survival [78].

Efforts to elucidate the prognostic value of different TP53 mutations have also led to the development of a novel computation approach termed the Evolutionary Action score of TP53-coding variants (EAp53; [80–82]). EAp53 stratifies HNSCC patients with tumors harboring TP53 missense mutations based on an estimated degree of risk assigned to each mutation. The foundational principles of this approach are based on previously identified TP53 “gain of function” mutations that enhanced cell transformation and chemotherapy resistance [83]. EAp53 assigns functional sensitivity to sequence variations based on evolutionary substitutions for every sequence position and calculates if substitutions correlate with larger or smaller phylogenetic divergences to determine “risk” [80, 82, 84]. HNSCC patients with p53 mutations classified using EAp53 as high-risk had significantly worse survival outcomes and reduced periods until distant metastases developed [80], as well

as increased resistance to chemotherapy [81]. As larger datasets with clinical annotations become available, it will be critical to refine and validate these models, and to determine if and how TP53 status is suitable to predict efficacy of different therapeutic interventions.

CDKN2A regulates cell cycle progression by blocking the activity of CCND1 (cyclin D1) and its associated kinases, CDK6 and CDK4, which phosphorylate and inactivate the tumor suppressor RB1 (Fig. 1b; [56, 85, 86]). Inactivation of the CDKN2A gene was found in 57 % of HPV- cases in the TCGA cohort [19]; however, other studies produced discordant values for genomic alterations of CDKN2A (ranging from 4 to 74 %; [20–22, 35, 36]; Table 1). Evaluation of CDKN2A status is somewhat complicated by the fact that the gene is GC-rich (> 60 % of bases are cytosine or guanine; [87]). Sequencing GC-rich regions can be problematic because of their higher melting temperature compared to GC-low regions, which is due to base stacking and more stable secondary structure [74, 75]. Methylation-associated inactivation of CDKN2A (further discussed below) is another important factor potentially complicating assessment of the function status of this gene [88–91]. Direct comparison of cases in the TCGA cohort with homozygous deletions or predicted inactivating mutations in CDKN2A versus wild type CDKN2A did not indicate a survival difference. However, as with TP53, subsequent refined analysis of CDKN2A status emphasized that the patient cohort with low mRNA expression of CDKN2A (RNA-seq: $z < 3$ -fold) did have reduced survival ($p = 0.037$; [56]). This observation is in accordance with other work that indicated improved survival for patients with p16-positive non-oro-pharyngeal squamous cell carcinoma [92]. Further emphasizing the importance of this signaling axis is the fact that CCND1 is the most frequently amplified gene in the TCGA cohort of HPV-HNSCC cases, detected in 31 % of cases (confirming earlier studies [19, 21, 22, 56]). Beck et al. reported that high RNA expression of CCND1 ($z > 2$ -fold) not only correlated with reduced survival in the TCGA dataset [19, 56], but also co-occurred frequently with CDKN2A deletions (co-occurrence ratio: 0.817). Cases harboring both, amplified CCND1 and deleted CDK2N2A had much worse prognosis than cases without these alterations [56].

In the TCGA cohort, EGFR was amplified in 12 % of HPV- cases [19], the same % of cases with EGFR amplification was reported by Seiwert et al. [21]. Seiwert et al. did not report significant incidence of alteration in HER2 (also known as ERBB2), ERBB3 and ERBB4 and the TCGA also only detected alterations of those genes in a small number of cases (4–6 %; [19]). Nevertheless, alterations in ERBB2 or ERBB3 have been directly linked to resistance to EGFR-targeted therapy and are thus of therapeutic relevance [93]. Mining of TCGA data

highlighted that RPPA expression of pHER2 correlated with expression of HER2, and both, pHER2 and HER2 expression correlated with protein expression of EGFR [93], providing some patient data in support of *in vivo* results in which dual kinase inhibition of EGFR and HER2 enhances response to cetuximab [94]. In addition, the RTKs FGFR1 and IGF1R were identified with activating mutations in 10 and 4 % of HPV- HNSCC, respectively, while no mutations of these kinases were identified in HPV+ HNSCC tumors (Fig. 1a and c). FGFR1 and IGF1R participate in a signaling network that includes EGFR and other ERBB family members (Fig. 1c), and both can contribute to resistance to EGFR-targeted therapeutics, the only type of targeted therapy approved for HNSCC [73, 95, 96]. Functioning downstream of these RTKs, the GTPase HRAS was almost exclusively altered in HPV- HNSCC (5 %), propagates pro-proliferation and pro-survival signaling via the BRAF-MEK-ERK axis, and provides alternative input to activate PI3K (Fig. 1c; [97–99]).

For two additional genes, AJUBA and FAT1, almost all detected alterations were found in HPV- tumors (Fig. 1a and d). Both genes are involved in differentiation and are linked to the NOTCH/CTNBN1 signaling pathway as negative regulators [19, 100, 101]. The scaffolding protein AJUBA, inactivated in 7 % of HPV- cases (0 % of HPV+ tumors), has also been implicated in interactions with Aurora-A kinase (AURKA), a critical regulator of mitosis [102]. AURKA is overexpressed in a significant percentage (7 %) of HNSCC cases and correlated with diminished survival in an analysis of provisional TCGA data (significant overlap with the published TCGA dataset; [19, 103]). FAT1 is a member of the cadherin-like protein family and has been described as a suppressor of cancer cell growth based on a role in binding to and antagonizing CTNBN1 [100]. FAT1 had previously been shown to be mutated in roughly 7 % of 60 head and neck tumors [100], but was detected to be inactivated (missense/truncating mutations and homozygous deletions) in a much greater percentage of HPV- cases (32 %; versus inactivated in only 3 % of HPV+ cases; Fig. 1a and d) analyzed by the TCGA network [19]. The discrepancy may be due to a number of reasons, including differences in sample processing, determination of HPV status, demographic factors, different acquisition platforms, and differently constructed analytical pipelines.

The TCGA analysis also identified a set of less well studied alterations associated with oxidative stress, specifically involving CUL3, KEAP1 and NFE2L2 [19]. KEAP1 and NFE2L2 were exclusively altered in HPV- HNSCC (Fig. 1a). KEAP1 was inactivated in 5 % of cases and NFE2L2 was activated in 14 % of cases. Functionally, NFE2L2 is a transcription factor that regulates antioxidant and stress-responsive genes [104]. KEAP1

complexes with the E3 ligase CUL3 (inactivated in 6 % of HPV- cases) to polyubiquitinate NFE2L2 [105]; thus, disruption of canonical KEAP1-CUL3 function promotes NFE2L2 activity [19]. Intriguingly, in lung cancer, a NFE2L2-centric gene signature has been proposed as a valuable prognostic biomarker [106]. This may be relevant because significant molecular similarities between HNSCC and lung squamous cell cancers (SQCC) exist [19, 43, 48, 107], including shared dysregulation of KEAP1 and NFE2L2. Secondary analysis of the TCGA dataset indeed revealed that DNA level alterations of any member of the KEAP1/CUL3/RBX1 complex correlated with significantly reduced survival (median survival of ~35 months versus ~72 months; [108]).

Lastly, the TCGA network detected co-amplification of chromosome regions 11q13 and 11q22. Found within region 11q22, an amplicon previously described in lung, esophageal and cervical cancer [109–111], are the coding sequences for BIRC2 and YAP1. BIRC2 encodes c-IAP1 and is a member of the inhibitor-of-apoptosis family [112]. Functionally, BIRC2 inhibits caspase activity, including the activity of CASP8 [112], and it has been shown that BIRC2 plays an important role in the ubiquitination and degradation of TRAF3 (tumor necrotizing factor receptor-associated factor 3), a negative regulator of NF- κ B activity [113]. BIRC2 is more commonly altered in HPV- HNSCC (7 % of cases versus 3 % of cases in HPV+ HNSCC) and, as would have been predicted based on functionality, CASP8 was also frequently detected as inactivated through mutations or homozygous deletion (11 % of HPV- cases; Fig. 1a). YAP1 is a proto-oncogenic transcription factor downstream of BIRC2 and associated with the Hippo pathway [114]. Amongst cancers analyzed by the TCGA, HNSCC had the fifth highest incident of amplified YAP1 (6.3 % of cases). Interestingly, a recent study found that YAP1 amplification strongly correlated with resistance to cetuximab *in vitro* [115], which may reflect YAP1 associated upregulation of the EGFR ligand amphiregulin; further investigations are needed to fully uncover the precise mechanism of this type of resistance [115, 116]. Amplification of region 11q13 includes the region encoding the Fas-associated death domain gene (FADD; established as frequently overexpressed in HNSCC [117] and found to be amplified in 32 % of HPV- cases analyzed by the TCGA [19]). Importantly, FADD has been implicated in increased lymph node metastasis in HNSCC [117].

HPV-Positive HNSCC

At the molecular level, HPV+ carcinomas significantly differ from HPV- cases, highlighted in great detail by the TCGA network and others [10, 19, 21, 27, 92]. A significant limitation of the TCGA study is the fact that only

36 HPV+ cases were analyzed [19], a limitation partially compensated for by the work of other groups (Table 1; [21, 23, 34, 118]). Further analysis of additional tumors is clearly needed; however, some conclusions can be made in spite of the limited numbers of cases.

HPV+ HNSCC is defined by infection of tumor cells with HPV. HPV DNA can exist either integrated into the human genome or in a nonintegrated form [34, 119]. Upon infection, the HPV genome (8 kb) is first amplified as extrachromosomal circular elements (episomes), some of which may subsequently integrate into one or more location within the host genome [119]. It has been reported that HPV integration sites are randomly distributed throughout the genome [120]. In one study, analysis of 35 of the 36 HPV+ TCGA HNSCC cases identified HPV integration in 25 cases and uncovered distinct gene expression and methylation patterns for HPV integrated versus non-integrated HNSCC, suggesting different pathogenic mechanisms [34]. Another study published similar results for essentially the same group of patients (36 HPV+ HNSCC), and detected HPV DNA integration in 24/36 cases [119]. The general observation regarding HPV integration is not unique to HNSCC, as HPV+ cervical cancers include HPV integrated and non-integrated cases [121]. Compared to episomal HPV DNA, transcripts derived from integrated viral DNA have been shown to be more stable and more strongly associated with increased proliferative capacity of affected cells [122]. It is likely that HPV integration, particularly if within or proximal to key cancer related genes, is important but not essential for the oncogenicity of HPV: the better understood oncogenic contribution of the virus is the production of different oncoproteins [34].

HPV oncoproteins include E6 and E7 (Fig. 1b; [122]), which perform complementary actions in eliminating negative regulators of the cell cycle. E6 binds p53 and targets this tumor suppressor for proteosomal degradation [123]. Tumor suppressor RB1 interacts with E7, which targets RB1 for degradation through association with the cullin 2-ubiquitin-ligase complex [124–126]. As E6 and E7 function through cell cycle dysregulation by eliminating RB1 and TP53, very few alterations in additional cell cycle regulators occur in HPV+ disease: inactivation of CDKN2A, or TP53, or overexpression of CDK6 or CCND1, occur seldom in HPV+ HNSCC. One exception is the transcription factor E2F1, which is normally inhibited by RB1 (Fig. 1b; [56]); it is the only cell cycle regulator identifier by the TCGA study as being predominantly altered in HPV+ cases (19 % activated via amplification of chromosome 20q11, seen in only in 2 % of HPV- HNSCC; Fig. 1a and b).

Also associated with HPV+ disease is the RTK FGFR3, which is activated in 11 % of cases through either mutation or a gene fusion event, and the aforementioned

TNF receptor associated factor TRAF3, inactivated in 22 % of cases (versus 1 % in HPV- disease). The FGFR3 fusion partner is TACC3, a protein critical for nucleation of microtubules at the centrosome [127], aberrantly expressed in some cancers and potentially targetable with small molecules [128]. A FGFR3-TACC3 fusion was first described in glioblastoma [129] and subsequently detected in nasopharyngeal carcinoma and other HNSCCs [130, 131]. This fusion event was detected in two of the 36 HPV+ and zero of the HPV- HNSCC TCGA cases [19]. Constitutive kinase activity of the FGFR3-TACC3 oncogene induces loss of mitotic fidelity and leads to aneuploidy [129, 131]. In cases where present, FGFR3-TACC3 appears to be tumor driving and patients are likely to disproportionately benefit from FGFR3 targeting therapy [131]. TRAF3 has mostly been studied in immunological processes and one of its main functions is regulation of NF κ B activity [132]. In subsequent studies in HNSCC, functional analysis of TRAF3 has suggested a tumor suppressive role of the gene when overexpressed, and increased cell proliferation in the context of depleted TRAF3 [133].

Tumor heterogeneity

HPV- and HPV+ HNSCC share one particularly challenging feature: tumor heterogeneity [24]. This aspect of tumor biology has garnered significant attention in recent years because of the immense clinical implications in terms of prognosis, drug resistance and precision medicine [134–136]. Extensive analysis of TCGA data indicates that it is of high relevance in HNSCC [24, 137]. One approach to study HNSCC heterogeneity is based on whole-exome sequencing (WES), which can be used to determine the fraction of total sequenced DNA that contains a given mutant allele: termed mutant-allele fraction (MAF). The width of MAF distribution, normalized to the median MAF value, constitutes the quantitative value of intra-tumor heterogeneity, and has been termed mutant-allele tumor heterogeneity (MATH; [137, 138]). Earlier work indicated that HPV- tumors had significantly higher heterogeneity than HPV+ tumors (though substantial even for HPV+ cases; [137]), which would be predicated based on the frequency of and genomic instability associated with TP53 mutations, increased age and continuous tobacco use [24].

Provocatively, in a ten-variable multivariate analysis of TCGA HNSCC data incorporating MATH scores, no prognostic significance of HPV status, N classification or TP53 mutational status was determined [138]. While the lack of significance in the multivariate analysis does not suggest irrelevance of the three parameters, it strongly suggests that further work is needed to unravel these variables and to determine how much each parameter

truly impacts disease progression and survival in the context of appreciated heterogeneity. For example, disruptive TP53 mutations [78] are strongly associated with higher intra-tumor heterogeneity as calculated by MATH (i.e., high MATH scores), and both TP53 mutational status and high MATH scores, based on univariate analysis, indicated reduced survival [78, 137].

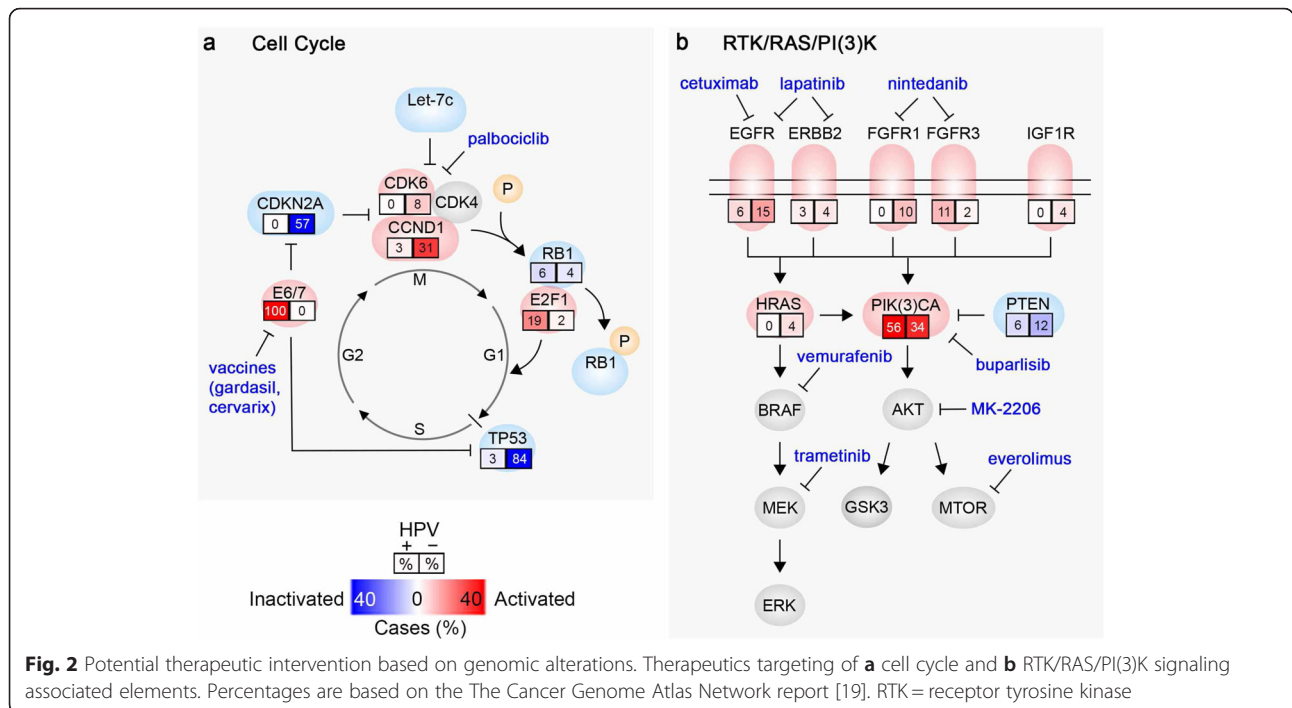
Additional innovative and detailed analysis by McGranahan et al. utilized TCGA datasets for nine tumor types, including HNSCC, to highlight important aspects of cancer evolution and clonality [24]. In order to determine if specific alterations were clonal (present in most/all tumor cells sequenced and therefore considered “early” mutations) or subclonal (present in a small fraction of cells and considered “late” mutations) McGranahan et al. used exome sequencing data and single-nucleotide polymorphism arrays to calculate the confidence interval of the cancer cell fraction (CCF; proportion of cancer cells harboring a given mutation; [139–141]) for a given mutation. A 95 % confidence interval of ≥ 1 was used to define clonal (“early”) mutations, and mutations with a confidence interval of less than 1 were defined as subclonal (“late”) mutations [24, 140–142]. In HNSCC, the majority of driver mutations were clonal, and CDKN2A and TP53 were identified as almost exclusively clonal. Based on the proportion of mutations, three mutational signatures (previously defined [25]) were identified for HNSCC: 1) a signature with C > T transitions at CpG sites associated with spontaneous deamination of methylated cytosines that strongly correlated with patient age at diagnosis and was most prevalently linked to “early” mutations; 2) a signature indicative of up-regulation of APOBEC cytosine deaminases [143], seen in both “early” and “late” mutations, although with significant prominence in “late” mutations; and 3) a signature associated with smoking induced mutations, seen predominantly with “early” mutations [24]. McGranahan et al. did not differentiate between HPV+ and HPV- cases, which future studies should do, particularly given that previous analysis of TCGA data detected evidence that HPV infection was strongly associated with APOBEC-mediated mutagenesis in HNSCC [144]. Furthermore, the same study suggested that APOBEC-mediated mutagenesis significantly contributes to helical domain E545K and E542K gain of function mutations in PIK3CA, one of the most frequently altered genes in HNSCC (32 out of 58 PIK3CA mutations in the TCGA cohort are E545K/E542K mutations; [19, 21, 144, 145]).

As an emerging concept for this disease, consideration of germline variants may be relevant to fully appreciate tumor heterogeneity. Recent analysis of TCGA data suggested that 15 % (44 out of 291 cases; incompletely congruent with the published TCGA dataset [19]) of HNSCC cases have rare germline truncations, including truncations in several genes important in the Fanconi

Anemia Pathway, specifically FANCA and FANCM, which are involved in DNA repair [146, 147]. FANCM mutations significantly correlated with increased somatic mutational frequency in the complete HNSCC cohort (mean age was 60.9 \pm 12.4 years; based on personal correspondence with authors), whereas, FANCA had a similar correlation with the frequency of somatic mutations, but specific in cases defined as younger age (mean age was 46.3 \pm 7.0 years) of onset (no indication regarding HPV status; [146]). Recent studies using murine models have implicated MYH9 as a gene that induces oral squamous cell carcinoma in the context of germline mutations or knockout [148, 149]. MYH9 encodes for non-muscle myosin II-A (NM II-A), best known for its roles as a cytoskeletal protein and during embryonic development [150]. Intriguingly, MYH9 may also act as a tumor suppressor, by regulating stabilization and nuclear retention of p53 [149]. MYH9 and MYH10 were mutated in 4 and 5 % of cases, respectively within the TCGA cohort of 279 patients [148]. No correlation with HPV status was detected. Success of future clinical efforts, particularly for targeted therapeutics, will likely heavily depend on consideration of heterogeneity and cancer evolution, guided by studies of spatio-temporal differences in genomic alterations, including presence or absence of germline mutations [134, 151].

Therapeutic insights

The majority of patients with HNSCC are treated with surgery and/or radiation and in some cases adjuvant chemotherapy [1, 3, 11, 27]. Treatment approaches for HPV- and HPV+ cases remain very similar [1]. However, because of the better prognosis and the younger age of onset associated with HPV+ disease, therapeutic de-intensification, currently only available as part of clinical trials, for the treatment of patients with HPV+ HNSCC is being actively explored [27, 152]. Thus far, the only targeted therapeutic approved to treat HNSCC is the monoclonal antibody cetuximab, designed to target the extracellular region of EGFR (Fig. 2; [153]). The clinical impact of cetuximab has been significant in some patients [154], but relatively modest overall [2, 73, 155]. Several small molecules, for example lapatinib (targeting EGFR and HER2; [156]), afatinib (targeting EGFR and HER2; [157]) and others (reviewed in [153]), have shown some promise in the treatment of HNSCC. Inter- and intra-disease heterogeneity are likely determining factors that have thus far held back greater success of available therapeutics, and represents one of the key challenges to overcome [19, 24, 151]. Consideration of a single gene, based on a single biopsy, does not seem sufficient to maximize therapeutic interventions [73]. For example, consideration of EGFR expression and/or amplification does not correspond with response to EGFR inhibitors



[107]. Data provided by the TCGA and others suggest that targeting EGFR may not be efficacious in the context of extensively altered parallel or downstream signaling components, including cell cycle regulators, due to overlapping functional contributions [73, 158].

Figure 2 summarizes potentially promising targets other than EGFR, based on available genomics data. The drugs shown in Fig. 2 are examples of drugs currently in clinical development for the treatment of HNSCC; recent reviews provide more complete lists of available drugs for each target [3, 159–161]. The near universality of cell cycle dysregulation in HNSCC strongly recommends investigation of CDK inhibitors [19, 56]. HPV-HNSCC with functional CDKN2A and high levels of phosphorylated RB1 may present the ideal molecular background for effective treatment with CDK4/6 inhibitors (Fig. 2; [56]). Furthermore, therapeutic targeting of aberrant cell cycle activity may partially circumvent the challenge presented by heterogeneity, given that clonal status analysis of the TCGA HNSCC cohort indicated that genes associated with cyclin-dependent kinases have 0 % of mutations arise in subclonal populations [24], which suggests that cell cycle alterations arise early during tumor development and are present in most if not all tumor cells. A large number of CDK inhibitors are currently in development [162] and the possibility of RB1 phosphorylation status as a response predictive biomarker is encouraging [56]. PI3K [NCT01816984], FGFR [NCT02558387], BRAF [NCT01286753], MEK [NCT01553851], AKT [NCT01349933] and mTOR [NCT010

51791] are further targets of potential therapeutic relevance (Fig. 2b; [102, 103, 163, 164]). Additional promising pre-clinical work has explored Second Mitochondria-derived Activator of Caspases (SMAC)-mimetics, antagonists of inhibitors of apoptosis, which seem particularly effective against HNSCC models with FADD/BIRC2 alterations [165, 166]; particularly meaningful considering the aforementioned high incident of FADD/BIRC2 alterations in HNSCC (Table 1).

The perhaps most exciting recent development in the treatment of cancer is immunotherapy [167]. Immunotherapy, specifically checkpoint blockade, has been tremendously successful in some cases of non-small cell lung cancer [168, 169], malignant melanoma [170] and other cancers [171, 172]. Checkpoint inhibitors seem to be particularly effective against tumors with high rates of mutation, which suggests that a subpopulation of patients with HNSCC would benefit from this type of therapy. Furthermore, HNSCC appears to be an immunosuppressive disease commonly associated with lymphopenia [173, 174] and in a few cases (7 % of HPV- and 11 % of HPV+ HNSCC in the TCGA cohort) presenting with specific mutations in HLA alleles and the antigen processing machinery to reduce tumor immunodetection [19]. A substantial number of clinical trials are currently exploring the applicability of immunotherapy for the treatment of HNSCC, with primary focus on immune checkpoint blockade via CTLA-4 and PD1 [12]. In brief, CTLA-4 and PD1 are expressed by T-cells and function as negative regulators of T-cell activity, a

process required for normal immunologic homeostasis. Tumor cells frequently engage CTLA-4 or PD1 to modulate T-cell activity and escape immunodetection [172, 175]. Immune checkpoint blockade inhibits interaction of tumor cells with CTLA-4 or PD1; thus, blocking inactivation of T-cells [175]. Regarding HNSCC, several phase III studies are currently exploring the utility of checkpoint inhibitors; specifically, the humanized monoclonal PD-1 specific antibody pembrolizumab [NCT02564263, NCT02358031, NCT02252042], recently approved for the treatment of melanoma and lung cancer (two cancer types with high mutational burden; [25, 26, 176–178]) and tremelimumab (fully human antibody against CTLA-4) with or without durvalumab (Fc optimized monoclonal antibody against the PD1 ligand 1; NCT02551159). Initial results are expected to be published in the near future [12]. It will be important to determine if distinct molecular lesions found in HNSCC, as summarized above, are prognostic for response to these new treatments. In the case of immunotherapy, considerations beyond the tumor may also be particularly important; for example, early laboratory studies have shown that the composition of the intestinal microbiota significantly impacts the efficacy of CTLA-4/PD-1 inhibitors [179, 180].

Methylation in HNSCC

Future endeavors are likely to include more extensive elucidation of the role of DNA methylation in HNSCC, in part to substantiate publications based on the TCGA dataset. DNA methylation is important in the regulation of gene expression, and aberrant methylation has been described for essentially all cancer types and as a critical aspect of cancer genomics [181, 182]. Previously published work suggests that HPV+ HNSCC has significantly differentiated CpG island methylation compared to HPV- cases, reflecting the notion that HPV+ and HPV- HNSCC are distinct diseases on the genomic, transcriptomic and methylomic level [183–185]. Comparative analyses of available HPV+ TCGA cases revealed specific hypermethylated regions downstream of CDKN2A, which correlated with increased transcription of CDKN2A variant p14 (ARF; [184]). CDKN2A is also frequently methylated (23–67 % of cases; [91]) to silence expression of this tumor suppressor [88, 90, 186]; although, degree of methylation and expression changes can vary significantly among individual tumors [187]. The mechanistic and clinical ramifications of this observation are not yet understood. Another study of HPV+ HNSCC reported that a promoter methylation signature of 5 genes, three with high methylation (GATA4, GRIA4, IRX4) and two with low methylation (ALDH1A2 and OSR2), correlated strongly with improved survival [188].

The signature was validated across multiple cohorts. Methylation patterns in HPV+ HNSCC are significantly distinct for cases with integrated HPV DNA and episomal DNA [34, 119], a potentially important factor not always considered.

Interestingly, prominent differential methylation of three members of the zinc finger gene family, ZNF14, ZNF160 and ZNF420, has been identified as suitable to detect HNSCC with 100 % specificity in primary tissue and saliva samples; subsequently, the three ZNF methylation signature was validated using the 273 TCGA cohort [185]. For most of the methylomics driven studies of HNSCC [183, 185, 188, 189], few cases were analyzed (particularly for HPV+ cases) and additional work is needed to better understanding and interpret the various methylation patterns. How methylomics data is going to be integrated into clinical practice for HNSCC remains to be seen, although prognostic and diagnostic potential of such information is apparent in some cancer types [181, 185, 190]. No DNA methylation markers for HNSCC have been accepted for clinical use to date [185].

Conclusions

Detailed profiling of HNSCC by the TCGA network and other research groups has greatly enhanced our understanding of this malignancy. First and foremost, the composite results have highlighted the tremendous inter- and intra-tumor heterogeneity, complicated by the increasing incidence of HPV-associated tumors. Efforts have started to focus on classifying tumors based on molecular profiles [191–193]; however, inroads in terms of improved survival have not substantially materialized yet. The next phase is likely to require multi-platform analysis of many more HPV- and HPV+ tumors, ideally sufficient to cover each anatomic site to enable actionable conclusions. In parallel, laboratory research and clinical trials have to continue to provide data that can guide therapeutic strategies based on molecularly defined parameters and higher-order interactions. Progress continues to be made and the status quo for patients with HNSCC is likely to continue to improve over the next decade.

The greatest potential therapeutic advantage to come from the detailed parsing of HNSCC heterogeneity is advanced and eventually precise treatment with immunotherapy. For example, consistent identification of tumors with highly immunogenic alterations would significantly help guide therapeutic decision-making [194]. Immunotherapy has been remarkably successful against many types of cancer, with particularly striking successes against other carcinogen-associated cancers, such as lung cancer [169, 195] and melanoma [170, 196]. High mutation burden, common for many sub-types of HNSCC [19, 24, 25], and carcinogen-associated genomic

profiles seems to correlate with higher efficacy of immunotherapy [194, 197]. Leveraging and advancing current knowledge to optimize selection of HNSCC cases for treatment with immunotherapy should be a top priority and could greatly enhance the many ongoing clinical trials [12]. The perhaps most promising approach to eradicate HPV+ HNSCC is extended use of the available vaccine, which currently appears to be successful in reducing rates of cervical cancer [198, 199] and would presumably be as successful in reducing the rate of HPV+ HNSCC.

Abbreviations

AJUBA: protein coding gene located at 14q11.2; adaptor/scaffolding protein; AKT: also known as AKT1, v-akt murine thymoma viral oncogenes homolog 1; codes for serine-threonine protein kinase and is located at 14q32.32; ALDH1A2: aldehyde dehydrogenase 1 family member A2; enzyme that catalyzes the synthesis of retinoic acid from retinaldehyde; APOBEC: apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; family of C-to-U editing cytidine deaminase enzymes located on chromosome 22; AURKA: aurora kinase A; protein is involved in cell cycle-regulation and microtubule formation; located at 20q13; BEZ-235: ATP-competitive inhibitor; imidazoquinoline derivative targeting PI3K/mTOR; also known as dactolisib; BIRC2: baculoviral IAP repeat containing 2; member of a family of proteins involved in inhibiting cell death; located at 11q22; BRAF: B-Raf proto-oncogene; member of the raf/mil family of serine/threonine kinases; located at 7q34; C > T transition: a point mutation that changes the pyrimidine thymine to the pyrimidine cytosine; CASP8: caspase 8, apoptosis-related cysteine peptidase; involved in programmed cell death; located at 2q33-q34; CCF: cancer cell fraction; used to infer the size of a subpopulation affected by somatic mutations from genomic data; CCND1: cyclin D1; member of the cyclin family associated with the regulation of CDKs; located at 11q13; CDK4: cyclin-dependent kinase 4; catalytic subunit of the protein kinase complex required for G1 cell cycle progression; located at 12q14; CDK6: cyclin-dependent kinase 6; catalytic subunit of the protein kinase complex required for G1 cell cycle progression; located at 7q21-q22; CDKN2A: cyclin-dependent kinase inhibitor 2A; codes for p16; inhibitor of CDK4; at least three alternatively spliced protein coding variants have been reported; located at 9p21; chromosome p: *petit*; shorter arm of the chromosome; chromosome q: longer arm of the chromosome; CNA: copy number alteration; gains or losses of large segments of the genome; CpG: cytosine and guanine separated by exactly one phosphate; amiable to methylation; CSMD1: CUB and Sushi multiple domains 1; located at 8p23.2; CTLA4: cytotoxic T-lymphocyte-associated protein 4; immunoglobulin; transmits inhibitory signal to T-cells; located at 2q33; CTNBN1: catenin beta 1; part of adherens junctions and signaling; located at 3p21; CUL3: cullin 3; component of an E3 ubiquitin ligase complex; located at 2q36.2; DNA: deoxyribonucleic acid; E2F1: E2F transcription factor 1; involved in cell cycle control; located at 20q11.2; E6: transforming protein; targeted p53; associated with human papillomavirus infection; E7: transforming protein; targets RB1; associated with human papillomavirus infection; EAp53: Evolutionary Action score of TP53-coding variants; computational approach to estimate the risk of various TP53 mutations in order to predict survival and treatment response; EGFR: epidermal growth factor receptor; member of the ERBB family of receptor tyrosine kinases; located at 7p12; EML4-ALK: fusion gene composed of echinoderm microtubule-associated protein-like 4 and anaplastic lymphoma kinase; chromosome 2p inversion; ERBB2: erb-b2 receptor tyrosine kinase 2; also known as HER2 or NEU; no ligand-binding domain; located 17q12; ERBB3: erb-b2 receptor tyrosine kinase 3; also known as HER3; no active kinase domain; located at 12q13; ERBB4: erb-b2 receptor tyrosine kinase 4; also known as HER4; located at 2q33.3-q34; ERK: officially known as MAPK1; mitogen-activated protein kinase 1; member of the MAP kinase family; located at 22q11.21; FADD: Fas associated via death domain; adaptor protein that interacts with surface receptors to mediate apoptosis; located at 11q13.3; FAT1: FAT atypical cadherin 1; member of the cadherin superfamily; located at 4q35; FGFR1: fibroblast growth factor receptor 1; receptor tyrosine kinase; located at 8p11.23-p11.22; FGFR3: fibroblast growth factor receptor 3;

receptor tyrosine kinase; located at 4p16.3; FHIT: fragile histidine triad; codes for triphosphate hydrolase required for purine metabolism; located at 3q14.2; G1: gap 1 phase; first phase of the cell cycle and part of interphase; GATA4: GATA binding protein 4; member of the GATA family of zinc-finger transcription factors; located at 8p23.1-p22; GEO: Gene Expression Omnibus; public genomic data repository; GISTIC: Genomic Identification of Significant Targets in Cancer; algorithm designed to identify likely driver somatic copy-number alterations by evaluating the amplitude and frequency of events; GRIA4: glutamate receptor, ionotropic, AMPA 4; AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate sensitive glutamate receptor; subject to RNA editing; located at 11q22; GTPase: family of enzymes that hydrolyze guanosine triphosphate (GTP); important for signal transduction in cancer cells; HLA: human leukocyte antigen; encode proteins essential to the immune system; chromosome 6; HNSCC: head and neck squamous cell carcinoma; HPV: human papillomavirus; DNA virus; HPV subtype 16 is most commonly associated with HNSCC; HPV-16: high-risk human papillomavirus subtype and the subtype most commonly associated with cervical, anal and oropharyngeal cancer; HRAS: Harvey rat sarcoma viral oncogenes homolog; member of the Ras oncogenes family with intrinsic GTPase activity; located at 11p15.5; IGF1R: insulin like growth factor 1 receptor; tyrosine kinase receptor; located at 11q26.3; IHC: immunohistochemistry; antibody-based tissue staining; IRX4: Iroquois homeobox 4; located at 5p15.3; *ISH*: *in situ* hybridization; uses labeled complementary DNA or RNA to localize specific DNA or RNA in tissue; KEAP1: kelch like ECH associated protein 1; interacts with NFE2L2 in a redox-sensitive manner; located at 19p13.2; let-7c: MIRLET7C also known as microRNA let-7c; short non-coding RNA involved in post-translational regulation of genes; located at 21q21.1; MAF: mutant-allele fraction; fractional of total sequenced DNA with a given mutant allele based on whole-exome sequencing; MATH: mutant-allele tumor heterogeneity; width of the mutant-allele fraction distribution normalized to the median MAF value; MEK: mitogen-activated protein kinase kinase; group of kinases also known as MAP2K; MAPKK or MKK; miRNA: micro RNA; small non-coding RNA molecule; mRNA: messenger ribonucleic acid; mTOR: mechanistic target of rapamycin; serine/threonine phosphatidylinositol kinase-related kinase; located at 1p36.2; MYC: v-myc avian myelocytomatosis viral oncogenes homolog; nuclear phosphoprotein and transcription factor; located at 8q24.21; MYH9: Nonmuscle Myosin Heavy Chain II-A (also known as NMHC-II-A); cytoskeleton protein; N classification: part of the TNM classification of malignant tumours staging notation system; describes cancer positive regional lymph nodes; ranges from N0 (tumor cells absent from regional lymph nodes) to N3 (tumor cells detected in distant lymph nodes); NFE2L2: transcription factor; regulates expression of genes with antioxidant response elements; located at 2q31; NFKB: NFKB1; nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; transcription regulator; located at 4q24; NOTCH: highly conserved signaling pathway; may also refer to NOTCH1; a type 1 single pass transmembrane receptor; located at 9q34.3; NPC: nasopharyngeal cancer; originates in the upper part of the throat behind the nose and near the base of the skull; OSCC: oral squamous cell carcinoma; OSR2: odd-skipped related transcription factor 2; located at 8q22.2; p14(ARF): alternative reading frame protein product of the CDKN2A locus; inhibits the E3 ubiquitin protein ligase MDM2 (p53 degradation); PCR: polymerase chain reaction; PD-1: PDCD1; programmed cell death 1; member of the immunoglobulin superfamily expressed on pro-B and T cells; located at 2q37.3; PI(3)K: phosphatidylinositol-4,5-bisphosphate 3-kinase; family of intracellular enzymes; PI3KCA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; codes for p110 α ; the catalytic subunit; located at 3q26.3; PLK1: polo-like kinase 1; serine/threonine kinase; highly expressed during mitosis; located at 16p12.2; RB1: retinoblastoma 1; negative regulator of the cell cycle; located at 13q14.2; RNA-Seq: RNA sequencing; next-generation sequencing to detect and quantify RNA; RPPA: reverse phase protein array; high-throughput antibody-based protein detection technique; RTK: receptor tyrosine kinases; large family of transmembrane tyrosine kinases; S: synthesis phase; phase of the cell cycle during which DNA is replicated; follows G1 and precedes G2; SOX2: SRY-box 2; transcription factor; located at 3q26.3-q27; SQCC: squamous cell carcinomas; TACC3: transforming, acidic coiled-coil containing protein 3; motor spindle protein; located at 4p16.3; TCGA: the Cancer Genome Atlas; TERT: telomerase reverse transcriptase; ribonucleoprotein polymerase; maintains telomere ends; located at 5p15.33; TP53: tumor protein p53; transcription factor and tumor suppressor; located at 17p13.1; TP63: tumor protein p63; p53 family transcription factor; located at 3q28; TP73: tumor protein p73; member of the p53 family of transcription

factors; located at 1p36.3; TRAF3: TNF receptor associated factor 3; member of the TNF receptor associated factor protein family; located at 14q32.32; WES: whole exome sequencing; technique for sequencing protein-coding genes; YAP1: Yes associated protein 1; nuclear effector of the Hippo pathway; located at 11q13; ZNF14: zinc finger protein 14; located at 19p13.11; ZNF160: zinc finger protein 160; located at 19q13.42; ZNF420: zinc finger protein 420; located at 19q13.12; Δ Np63a: isoform of the p53 homologue TP63 that lacks (Δ N) the transactivation domain.

Acknowledgements

The authors were supported by U54 CA149147, R21 CA181287, R21 CA191425 and P50 CA083638 from the NIH (to EAG), the Ruth L. Kirschstein NRSA F30 fellowship (F30 CA180607) from the NIH (to TNB) and NCI Core Grant P30 CA006927 (to Fox Chase Cancer Center).

Authors' contributions

Conception and design: TNB. Collection and assembly of data: TNB. Data analysis and interpretation: Both authors. Manuscript writing: Both authors. Both authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Program in Molecular Therapeutics, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111, USA. ²Program in Molecular and Cell Biology and Genetics, Drexel University College of Medicine, Philadelphia, PA 19129, USA.

Received: 22 January 2016 Accepted: 9 May 2016

Published online: 03 June 2016

References

- Pfister DG, Spencer S, Brizel DM, Burtneess B, Busse PM, Caudell JJ, Cmelak AJ, Colevas AD, Dunphy F, Eisele DW et al. Head and Neck Cancers, Version 1.2015 Featured Updates to the NCCN Guidelines. *J Natl Compr Canc Netw*. 2015;13(7):847–56.
- Burtneess B, Golemis EA. Overview: the pathobiology of head and neck cancer. In: Burtneess B, Golemis EA, editors. *Molecular determinants of head and neck cancer*. 1st ed. New York: Springer New York; 2014. p. 1–5.
- Dillon MT, Harrington KJ. Human papillomavirus-negative pharyngeal cancer. *J Clin Oncol*. 2015;33(29):3251–61.
- Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, Rosenberg PS, Bray F, Gillison ML. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *J Clin Oncol*. 2013;31(36):4550–9.
- Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of human papillomavirus-positive head and neck squamous cell carcinoma. *J Clin Oncol*. 2015;33(29):3235–42.
- Hashibe M, Brennan P, Benhamou S, Castellsague X, Chen C, Curado MP, Dal Maso L, Daudt AW, Fabianova E, Fernandez L et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *J Natl Cancer Inst*. 2007;99(10):777–89.
- Mehanna H, Beech T, Nicholson T, EH-Hariry I, McConkey C, Paleri V, Roberts S. Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer—systematic review and meta-analysis of trends by time and region. *Head Neck*. 2013;35(5):747–55.
- Chung CH, Bagheri A, D'Souza G. Epidemiology of oral human papillomavirus infection. *Oral Oncol*. 2014;50(5):364–9.
- Maxwell JH, Grandis JR, Ferris RL. HPV-Associated Head and Neck Cancer: unique features of epidemiology and clinical management. *Annu Rev Med*. 2016;67:91–101.
- Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, Westra WH, Chung CH, Jordan RC, Lu C et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *New Engl J Med*. 2010;363(1):24–35.
- Ang KK, Chen A, Curran Jr WJ, Garden AS, Harari PM, Murphy BA, et al. Head and neck carcinoma in the United States: first comprehensive report of the Longitudinal Oncology Registry of Head and Neck Carcinoma (LORHAN). *Cancer*. 2012;118(23):5783–92.
- Ferris RL. Immunology and immunotherapy of head and neck cancer. *J Clin Oncol*. 2015;33(29):3293–304.
- Huang SH, Xu W, Waldron J, Siu L, Shen X, Tong L, Ringash J, Bayley A, Kim J, Hope A et al. Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas. *J Clin Oncol*. 2015;33(8):836–45.
- O'Rourke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. *Oral Oncol*. 2012;48(12):1191–201.
- Garraway LA, Lander ES. Lessons from the cancer genome. *Cell*. 2013;153(1):17–37.
- Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz Jr LA, Kinzler KW. Cancer genome landscapes. *Science*. 2013;339(6127):1546–58.
- Cancer Genome Atlas Research N, Weinstein JN, Collisson EA, Mills GB, Shaw KR, Ozenberger BA, Ellrott K, Shmulevich I, Sander C, Stuart JM. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet*. 2013;45(10):1113–20.
- Hoadley KA, Yau C, Wolf DM, Cherniack AD, Tamborero D, Ng S, Leiserson MDM, Niu BF, McLellan MD, Uzunangelov V et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell*. 2014;158(4):929–44.
- Cancer Genome Atlas N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*. 2015;517(7536):576–82.
- Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang J, Wang J et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science*. 2011;333(6046):1154–7.
- Seiwert TY, Zuo ZX, Keck MK, Khattri A, Pedamallu CS, Stricker T, Brown C, Pugh TJ, Stojanov P, Cho J et al. Integrative and Comparative Genomic Analysis of HPV-Positive and HPV-Negative Head and Neck Squamous Cell Carcinomas. *Clin Cancer Res*. 2015;21(3):632–41.
- Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrence MS, Sougnez C, McKenna A et al. The mutational landscape of head and neck squamous cell carcinoma. *Science*. 2011;333(6046):1157–60.
- Lui VW, Hedberg ML, Li H, Vangara BS, Pendleton K, Zeng Y, Lu Y, Zhang Q, Du Y, Gilbert BR et al. Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. *Cancer Discov*. 2013;3(7):761–9.
- McGranahan N, Favero F, de Bruin EC, Birkbak NJ, Szallasi Z, Swanton C. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. *Sci Transl Med*. 2015;7(283):283ra54.
- Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Borresen-Dale AL et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500(7463):415–21.
- Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. *Nat Genet*. 2013;45(10):1127–33.
- Bhatia A, Burtneess B. Human papillomavirus-associated oropharyngeal cancer: defining risk groups and clinical trials. *J Clin Oncol*. 2015;33(29):3243–50.
- Vokes EE, Agrawal N, Seiwert TY. HPV-Associated Head and Neck Cancer. *J Natl Cancer Inst*. 2015;107(12):djv344.
- Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. *Nat Rev Cancer*. 2011;11(1):9–22.
- McLaughlin-Drubin ME, Crum CP, Munger K. Human papillomavirus E7 oncoprotein induces KDM6A and KDM6B histone demethylase expression and causes epigenetic reprogramming. *Proc Natl Acad Sci U S A*. 2011;108(5):2130–5.
- Munger K, Jones DL. Human papillomavirus carcinogenesis: an identity crisis in the retinoblastoma tumor suppressor pathway. *J Virol*. 2015;89(9):4708–11.
- Agger K, Cloos PA, Rudkjaer L, Williams K, Andersen G, Christensen J, Helin K. The H3K27me3 demethylase JMJD3 contributes to the activation of the INK4A-ARF locus in response to oncogene- and stress-induced senescence. *Genes Dev*. 2009;23(10):1171–6.
- Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, Viscidi R. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst*. 2008;100(6):407–20.
- Parfenov M, Pedamallu CS, Gehlenborg N, Freeman SS, Danilova L, Bristow CA, Lee S, Hadjipanayis AG, Ivanova EV, Wilkerson MD et al. Characterization

- of HPV and host genome interactions in primary head and neck cancers. *Proc Natl Acad Sci U S A*. 2014;111(43):15544–9.
35. Pickering CR, Zhang J, Yoo SY, Bengtsson L, Moorthy S, Neskey DM, Zhao M, Ortega Alves MV, Chang K, Drummond J et al. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov*. 2013;3(7):770–81.
 36. Pickering CR, Zhang JX, Neskey DM, Zhao M, Jasser SA, Wang JP, Ward A, Tsai CJ, Alves MVO, Zhou JH et al. Squamous cell carcinoma of the oral tongue in young Non-smokers is genomically similar to tumors in older smokers. *Clin Cancer Res*. 2014;20(14):3842–8.
 37. Lin DC, Meng X, Hazawa M, Nagata Y, Varela AM, Xu L, Sato Y, Liu LZ, Ding LW, Sharma A et al. The genomic landscape of nasopharyngeal carcinoma. *Nat Genet*. 2014;46(8):866–71.
 38. Gao JJ, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun YC, Jacobsen A, Sinha R, Larsson E et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6(269):pl1.
 39. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401–4.
 40. Mermel CH, Schumacher SE, Hill B, Meyerson ML, Beroukhi R, Getz G. GISTIC2.0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome Biol*. 2011;12(4):R41.
 41. Masuda M, Yamada T. Signaling pathway profiling by reverse-phase protein array for personalized cancer medicine. *Biochim Biophys Acta*. 2015;1854(6):651–7.
 42. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets-update. *Nucleic Acids Res*. 2013;41(D1):D991–5.
 43. Hayes DN, Van Waes C, Seiwert TY. Genetic landscape of human papillomavirus-associated head and neck cancer and comparison to tobacco-related tumors. *J Clin Oncol*. 2015;33(29):3227–34.
 44. Walter V, Yin X, Wilkerson MD, Cabanski CR, Zhao N, Du Y, et al. Molecular subtypes in head and neck cancer exhibit distinct patterns of chromosomal gain and loss of canonical cancer genes. *PLoS One*. 2013;8(2):e56823.
 45. Tai SK, Lee JJ, Ang KK, El-Naggar AK, Hassan KA, Liu D, et al. Loss of FHIT expression in head and neck squamous cell carcinoma and its potential clinical implication. *Clin Cancer Res*. 2004;10(16):5554–7.
 46. Ma C, Quesnelle KM, Sparano A, Rao S, Park MS, Cohen MA, et al. Characterization CSMD1 in a large set of primary lung, head and neck, breast and skin cancer tissues. *Cancer Biol Ther*. 2009;8(10):907–16.
 47. Scholnick SB, Richter TM. The role of CSMD1 in head and neck carcinogenesis. *Genes Chromosomes Cancer*. 2003;38(3):281–3.
 48. Cancer Genome Atlas Research N. Comprehensive genomic characterization of squamous cell lung cancers. *Nature*. 2012;489(7417):519–25.
 49. Liu Z, Long XB, Chao C, Yan C, Wu QY, Hua SN, Zhang YJ, Wu AB, Fang WY. Knocking down CDK4 mediates the elevation of let-7c suppressing cell growth in nasopharyngeal carcinoma. *Bmc Cancer*. 2014;14:274.
 50. Liu N, Boohaker RJ, Jiang C, Boohaker JR, Xu B. A radiosensitivity MiRNA signature validated by the TCGA database for head and neck squamous cell carcinomas. *Oncotarget*. 2015;6(33):34649–57.
 51. Thorpe LM, Yuzugullu H, Zhao JJ. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nat Rev Cancer*. 2015;15(1):7–24.
 52. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer*. 2002;2(7):489–501.
 53. Elkabets M, Pazarentzos E, Juric D, Sheng Q, Pelosof RA, Brook S, et al. AXL mediates resistance to PI3K alpha inhibition by activating the EGFR/PKC/mTOR axis in head and neck and esophageal squamous cell carcinomas. *Cancer Cell*. 2015;27(4):533–46.
 54. Samuels Y, Diaz Jr LA, Schmidt-Kittler O, Cummins JM, DeLong L, Cheong I, et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell*. 2005;7(6):561–73.
 55. Halilovic E, She QB, Ye Q, Pagliarini R, Sellers WR, Solit DB, et al. PIK3CA mutation uncouples tumor growth and cyclin D1 regulation from MEK/ERK and mutant KRAS signaling. *Cancer Res*. 2010;70(17):6804–14.
 56. Beck TN, Kaczmar J, Handorf E, Nikonova A, Dubyk C, Peri S, et al. Phospho-T356RB1 predicts survival in HPV-negative squamous cell carcinoma of the head and neck. *Oncotarget*. 2015;6(22):18863–74.
 57. Brachmann SM, Hofmann I, Schnell C, Fritsch C, Wee S, Lane H, et al. Specific apoptosis induction by the dual PI3K/mTOR inhibitor NVP-BE2235 in HER2 amplified and PIK3CA mutant breast cancer cells. *Proc Natl Acad Sci U S A*. 2009;106(52):22299–304.
 58. Mohan S, Vander Broek R, Shah S, Eytan DF, Pierce ML, Carlson SG, et al. MEK Inhibitor PD-0325901 Overcomes Resistance to PI3K/mTOR Inhibitor PF-5212384 and potentiates antitumor effects in Human Head and Neck Squamous Cell Carcinoma. *Clin Cancer Res*. 2015;21(17):3946–56.
 59. Herzog A, Bian YS, Vander Broek R, Hall B, Coupar J, Cheng H, et al. PI3K/mTOR Inhibitor PF-04691502 Antitumor Activity Is Enhanced with Induction of Wild-Type TP53 in Human Xenograft and Murine Knockout Models of Head and Neck Cancer. *Clin Cancer Res*. 2013;19(14):3808–19.
 60. Rodon J, Dienstmann R, Serra V, Tabernero J. Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol*. 2013;10(3):143–53.
 61. Dotto GP. Crosstalk of Notch with p53 and p63 in cancer growth control. *Nat Rev Cancer*. 2009;9(8):587–95.
 62. Nicolas M, Wolfer A, Raj K, Kummer JA, Mill P, van Noort M, et al. Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet*. 2003;33(3):416–21.
 63. Duan L, Yao J, Wu X, Fan M. Growth suppression induced by Notch1 activation involves Wnt-beta-catenin down-regulation in human tongue carcinoma cells. *Biol Cell*. 2006;98(8):479–90.
 64. Dotto GP. Notch tumor suppressor function. *Oncogene*. 2008;27(38):5115–23.
 65. Okuyama R, Ogawa E, Nagoshi H, Yabuki M, Kurihara A, Terui T, et al. p53 homologue, p51/p63, maintains the immaturity of keratinocyte stem cells by inhibiting Notch1 activity. *Oncogene*. 2007;26(31):4478–88.
 66. Yang AN, Kaghad M, Wang YM, Gillett E, Fleming MD, Dotsch V, et al. p63, a p53 homologue at 3q27–29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities. *Mol Cell*. 1998;2(3):305–16.
 67. Wu G, Nomoto S, Hoque MO, Dracheva T, Osada M, Lee CC, et al. DeltaNp63alpha and TAp63alpha regulate transcription of genes with distinct biological functions in cancer and development. *Cancer Res*. 2003;63(10):2351–7.
 68. Westfall MD, Pietenpol JA. p63: Molecular complexity in development and cancer. *Carcinogenesis*. 2004;25(6):857–64.
 69. Melino G. p63 is a suppressor of tumorigenesis and metastasis interacting with mutant p53. *Cell Death Differ*. 2011;18(9):1487–99.
 70. Zangen R, Ratovitski E, Sidransky D. Delta Np63 alpha levels correlate with clinical tumor response to cisplatin. *Cell Cycle*. 2005;4(10):1313–5.
 71. Riese U, Dahse R, Fiedler W, Theuer C, Koscielny S, Ernst G, et al. Tumor suppressor gene p16 (CDKN2A) mutation status and promoter inactivation in head and neck cancer. *Int J Mol Med*. 1999;4(1):61–5.
 72. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res*. 1994;54(18):4855–78.
 73. Burtress B, Bauman JE, Galloway T. Novel targets in HPV-negative head and neck cancer: overcoming resistance to EGFR inhibition. *Lancet Oncol*. 2013;14(8):e302–9.
 74. Jensen MA, Fukushima M, Davis RW. DMSO and betaine greatly improve amplification of GC-rich constructs in de novo synthesis. *PLoS One*. 2010;5(6):e11024.
 75. Kozarewa I, Ning Z, Quail MA, Sanders MJ, Berriman M, Turner DJ. Amplification-free Illumina sequencing-library preparation facilitates improved mapping and assembly of (G + C)-biased genomes. *Nat Methods*. 2009;6(4):291–5.
 76. Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, et al. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med*. 2007;357(25):2552–61.
 77. Masica DL, Li S, Douville C, Manola J, Ferris RL, Burtress B, et al. Predicting survival in head and neck squamous cell carcinoma from TP53 mutation. *Hum Genet*. 2015;134(5):497–507.
 78. Gross AM, Orosco RK, Shen JP, Egloff AM, Carter H, Hofree M, et al. Multi-tiered genomic analysis of head and neck cancer ties TP53 mutation to 3p loss. *Nat Genet*. 2014;46(9):939–43.
 79. Song Y, Li L, Ou Y, Gao Z, Li E, Li X, et al. Identification of genomic alterations in esophageal squamous cell cancer. *Nature*. 2014;509(7498):91–5.
 80. Neskey DM, Osman AA, Ow TJ, Katsonis P, McDonald T, Hicks SC, et al. Evolutionary action score of TP53 identifies high-risk mutations associated with decreased survival and increased distant metastases in head and neck cancer. *Cancer Res*. 2015;75(7):1527–36.

81. Osman AA, Neskey DM, Katsonis P, Patel AA, Ward AM, Hsu TK, et al. Evolutionary action score of TP53 coding variants is predictive of platinum response in head and neck cancer patients. *Cancer Res.* 2015; 75(7):1205–15.
82. Katsonis P, Lichtarge O. A formal perturbation equation between genotype and phenotype determines the evolutionary action of protein-coding variations on fitness. *Genome Res.* 2014;24(12):2050–8.
83. Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. *Nat Rev Cancer.* 2009;9(10):701–13.
84. Lichtarge O, Wilkins A. Evolution: a guide to perturb protein function and networks. *Curr Opin Struc Biol.* 2010;20(3):351–9.
85. Weinberg RA. The retinoblastoma protein and cell cycle control. *Cell.* 1995; 81(3):323–30.
86. Burke JR, Hura GL, Rubin SM. Structures of inactive retinoblastoma protein reveal multiple mechanisms for cell cycle control. *Gene Dev.* 2012;26(11): 1156–66.
87. Wong SQ, Li J, Salemi R, Sheppard KE, Do H, Tothill RW, et al. Targeted-capture massively-parallel sequencing enables robust detection of clinically informative mutations from formalin-fixed tumours. *Sci Rep.* 2013;3:3494.
88. Demokan S, Chuang A, Suoglu Y, Uluhan M, Yalnz Z, Califano JA, et al. Promoter methylation and loss of p16INK4a gene expression in head and neck cancer. *Head Neck-J Sci Spec.* 2012;34(10):1470–5.
89. El-Naggar AK, Lai S, Clayman G, Lee JK, Luna MA, Goepfert H, et al. Methylation, a major mechanism of p16/CDKN2 gene inactivation in head and neck squamous carcinoma. *Am J Pathol.* 1997;151(6):1767–74.
90. Reed AL, Califano J, Cairns P, Westra WH, Jones RM, Koch W, et al. High frequency of p16 (CDKN2/MTS-1/INK4A) inactivation in head and neck squamous cell carcinoma. *Cancer Res.* 1996;56(16):3630–3.
91. Ha PK, Califano JA. Promoter methylation and inactivation of tumour-suppressor genes in oral squamous-cell carcinoma. *Lancet Oncol.* 2006;7(1): 77–82.
92. Chung CH, Zhang Q, Kong CS, Harris J, Fertig EJ, Harari PM, et al. p16 protein expression and human papillomavirus status as prognostic biomarkers of nonoropharyngeal head and neck squamous cell carcinoma. *J Clin Oncol.* 2014;32(35):3930–U3212.
93. Pollock NI, Grandis JR. HER2 as a therapeutic target in head and neck squamous cell carcinoma. *Clin Cancer Res.* 2015;21(3):526–33.
94. Quesnelle KM, Grandis JR. Dual kinase inhibition of EGFR and HER2 overcomes resistance to cetuximab in a novel in vivo model of acquired cetuximab resistance. *Clin Cancer Res.* 2011;17(18):5935–44.
95. Ratushny V, Astsaturov I, Burtness BA, Golemis EA, Silverman JS. Targeting EGFR resistance networks in head and neck cancer. *Cell Signal.* 2009;21(8):1255–68.
96. Marshall ME, Hinz TK, Kono SA, Singleton KR, Bichon B, Ware KE, et al. Fibroblast growth factor receptors are components of autocrine signaling networks in head and neck squamous cell carcinoma cells. *Clin Cancer Res.* 2011;17(15):5016–25.
97. Wellbrock C, Karasarides M, Marais R. The RAF proteins take centre stage. *Nat Rev Mol Cell Biol.* 2004;5(11):875–85.
98. Weber CK, Slupsky JR, Herrmann C, Schuler M, Rapp UR, Block C. Mitogenic signaling of Ras is regulated by differential interaction with Raf isozymes. *Oncogene.* 2000;19(2):169–76.
99. Logue JS, Morrison DK. Complexity in the signaling network: insights from the use of targeted inhibitors in cancer therapy. *Gene Dev.* 2012; 26(7):641–50.
100. Morris LGT, Kaufman AM, Gong YX, Ramaswami D, Walsh LA, Turcan S, et al. Recurrent somatic mutation of FAT1 in multiple human cancers leads to aberrant Wnt activation. *Nat Genet.* 2013;45(3):253–61.
101. Haraguchi K, Ohsugi M, Abe Y, Semba K, Akiyama T, Yamamoto T. Ajuba negatively regulates the Wnt signaling pathway by promoting GSK-3beta-mediated phosphorylation of beta-catenin. *Oncogene.* 2008;27(3):274–84.
102. Nikonova AS, Astsaturov I, Serebriiskii IG, Dunbrack RL, Golemis EA. Aurora A kinase (AURKA) in normal and pathological cell division. *Cell Mol Life Sci.* 2013;70(4):661–87.
103. Mehra R, Serebriiskii IG, Burtness B, Astsaturov I, Golemis EA. Aurora kinases in head and neck cancer. *Lancet Oncol.* 2013;14(10):e425–35.
104. Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev.* 2013;27(20):2179–91.
105. Cullinan SB, Gordan JD, Jin JO, Harper JW, Diehl JA. The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: Oxidative stress sensing by a Cul3-Keap1 ligase. *Mol Cell Biol.* 2004;24(19):8477–86.
106. Qian Z, Zhou T, Gurguis CI, Xu X, Wen Q, Lv J, et al. Nuclear factor, erythroid 2-like 2-associated molecular signature predicts lung cancer survival. *Sci Rep.* 2015;5:16889.
107. Hammerman PS, Hayes DN, Grandis JR. Therapeutic insights from genomic studies of head and neck squamous cell carcinomas. *Cancer Discov.* 2015; 5(3):239–44.
108. Martinez VD, Vucic EA, Thu KL, Pikor LA, Lam S, Lam WL. Disruption of KEAP1/CUL3/RBX1 E3-ubiquitin ligase complex components by multiple genetic mechanisms: association with poor prognosis in head and neck cancer. *Head Neck-J Sci Spec.* 2015;37(5):727–34.
109. Choschick M, Tabibzade AM, Giesecking F, Woelber L, Jaenicke F, Sauter G, et al. BIRC2 amplification in squamous cell carcinomas of the uterine cervix. *Virchows Arch.* 2012;461(2):123–8.
110. Imoto I, Tsuda H, Hirasawa A, Miura M, Sakamoto M, Hirohashi S, et al. Expression of cIAP1, a target for 11q22 amplification, correlates with resistance of cervical cancers to radiotherapy. *Cancer Res.* 2002;62(17):4860–6.
111. Imoto I, Yang ZQ, Pimkhaokham A, Tsuda H, Shimada Y, Imamura M, et al. Identification of cIAP1 as a candidate target gene within an amplicon at 11q22 in esophageal squamous cell carcinomas. *Cancer Res.* 2001;61(18): 6629–34.
112. Phillips AH, Schoeffler AJ, Matsui T, Weiss TM, Blankenship JW, Zobel K, et al. Internal motions prime cIAP1 for rapid activation. *Nat Struct Mol Biol.* 2014; 21(12):1068–74.
113. Vallabhapurapu S, Matsuzawa A, Zhang WZ, Tseng PH, Keats JJ, Wang HP, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappa B signaling. *Nat Immunol.* 2008;9(12):1364–70.
114. Stanger BZ. Quit your YAPing: a new target for cancer therapy. *Gene Dev.* 2012;26(12):1263–7.
115. Jerhammar F, Johansson AC, Ceder R, Welander J, Jansson A, Grafstrom RC, et al. YAP1 is a potential biomarker for cetuximab resistance in head and neck cancer. *Oral Oncol.* 2014;50(9):832–9.
116. Zhang JM, Ji JY, Yu M, Overholtzer M, Smolen GA, Wang R, et al. YAP-dependent induction of amphiregulin identifies a non-cell-autonomous component of the Hippo pathway. *Nat Cell Biol.* 2009;11(12):1444–U1134.
117. Pattje WJ, Melchers LJ, Slagter-Menkema L, Mastik MF, Schrijvers ML, Gibcus JH, et al. FADD expression is associated with regional and distant metastasis in squamous cell carcinoma of the head and neck. *Histopathology.* 2013; 63(2):263–70.
118. Lechner M, Frampton GM, Fenton T, Feber A, Palmer G, Jay A, et al. Targeted next-generation sequencing of head and neck squamous cell carcinoma identifies novel genetic alterations in HPV+ and HPV- tumors. *Genome Med.* 2013;5(5):49.
119. Khoury JD, Tannir NM, Williams MD, Chen YX, Yao H, Zhang JP, et al. Landscape of DNA virus associations across human malignant cancers: analysis of 3775 cases using RNA-Seq. *J Virol.* 2013;87(16):8916–26.
120. Wentzensen N, Vinokurova S, Doebertitz MV. Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. *Cancer Res.* 2004; 64(11):3878–84.
121. Hu Z, Zhu D, Wang W, Li W, Jia W, Zeng X, et al. Genome-wide profiling of HPV integration in cervical cancer identifies clustered genomic hot spots and a potential microhomology-mediated integration mechanism. *Nat Genet.* 2015;47(2):158–63.
122. Moody CA, Laimins LA. Human papillomavirus oncoproteins: pathways to transformation. *Nat Rev Cancer.* 2010;10(8):550–60.
123. Thomas M, Pim D, Banks L. The role of the E6-p53 interaction in the molecular pathogenesis of HPV. *Oncogene.* 1999;18(53):7690–700.
124. Huh K, Zhou XB, Hayakawa H, Cho JY, Libermann TA, Jin JP, et al. Human papillomavirus type 16 E7 oncoprotein associates with the cullin 2 ubiquitin ligase complex, which contributes to degradation of the retinoblastoma tumor suppressor. *J Virol.* 2007;81(18):9737–47.
125. Dyson N, Howley PM, Munger K, Harlow E. The human papilloma virus-16 E7-oncoprotein is able to bind to the retinoblastoma gene-product. *Science.* 1989;243(4893):934–7.
126. Chung CH, Gillison ML. Human papillomavirus in head and neck cancer: its role in pathogenesis and clinical implications. *Clin Cancer Res.* 2009;15(22): 6758–62.
127. Singh P, Thomas GE, Gireesh KK, Manna TK. TACC3 protein regulates microtubule nucleation by affecting gamma-tubulin ring complexes. *J Biol Chem.* 2014;289(46):31719–35.

128. Yao R, Kondoh Y, Natsume Y, Yamanaka H, Inoue M, Toki H, et al. A small compound targeting TACC3 revealed its different spatiotemporal contributions for spindle assembly in cancer cells. *Oncogene*. 2014;33(33):4242–52.
129. Singh D, Chan JM, Zoppoli P, Niola F, Sullivan R, Castano A, et al. Transforming Fusions of FGFR and TACC Genes in Human Glioblastoma. *Science*. 2012;337(6099):1231–5.
130. Wu YM, Su F, Kalyana-Sundaram S, Khazanov N, Ateeq B, Cao X, et al. Identification of targetable FGFR gene fusions in diverse cancers. *Cancer Discov*. 2013;3(6):636–47.
131. Yuan L, Liu ZH, Lin ZR, Xu LH, Zhong Q, Zeng MS. Recurrent FGFR3-TACC3 fusion gene in nasopharyngeal carcinoma. *Cancer Biol Ther*. 2014;15(12):1613–21.
132. Hacker H, Tseng PH, Karin M. Expanding TRAF function: TRAF3 as a tri-faced immune regulator. *Nat Rev Immunol*. 2011;11(7):457–68.
133. Zhang J, Chen H, Yang X, Guven E, Nussinov R, Chen Z, VanWaes C. Defective TRAF3 modulates alternative NF- κ B signaling and cytokine expression to promote cancer cell survival in HPV positive head and neck cancer (TUM10P.1049). *J Immunol*. 2015;194:211.30.
134. McGranahan N, Swanton C. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. *Cancer Cell*. 2015;27(1):15–26.
135. Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, et al. Toward understanding and exploiting tumor heterogeneity. *Nat Med*. 2015;21(8):846–53.
136. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012;366(10):883–92.
137. Mroz EA, Rocco JW. MATH, a novel measure of intratumor genetic heterogeneity, is high in poor-outcome classes of head and neck squamous cell carcinoma. *Oral Oncol*. 2013;49(3):211–5.
138. Mroz EA, Tward AM, Hammon RJ, Ren Y, Rocco JW. Intra-tumor genetic heterogeneity and mortality in head and neck cancer: analysis of data from the Cancer Genome Atlas. *Plos Med*. 2015;12(2):e1001786.
139. Landau DA, Carter SL, Getz G, Wu CJ. Clonal evolution in hematological malignancies and therapeutic implications. *Leukemia*. 2014;28(1):34–43.
140. Landau DA, Carter SL, Stojanov P, McKenna A, Stevenson K, Lawrence MS, et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell*. 2013;152(4):714–26.
141. Lohr JG, Stojanov P, Carter SL, Cruz-Gordillo P, Lawrence MS, Auclair D, et al. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. *Cancer Cell*. 2014;25(1):91–101.
142. Carter SL, Cibulskis K, Helman E, McKenna A, Shen H, Zack T, et al. Absolute quantification of somatic DNA alterations in human cancer. *Nat Biotechnol*. 2012;30(5):413–21.
143. Swanton C, McGranahan N, Starrett GJ, Harris RS. APOBEC enzymes: mutagenic fuel for cancer evolution and heterogeneity. *Cancer Discov*. 2015;5(7):704–12.
144. Henderson S, Chakravarthy A, Su X, Boshoff C, Fenton TR. APOBEC-mediated cytosine deamination links PIK3CA helical domain mutations to human papillomavirus-driven tumor development. *Cell Rep*. 2014;7(6):1833–41.
145. Zhao L, Vogt PK. Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci U S A*. 2008;105(7):2652–7.
146. Lu C, Xie M, Wendl MC, Wang J, McLellan MD, Leiserson MD, et al. Patterns and functional implications of rare germline variants across 12 cancer types. *Nat Commun*. 2015;6:10086.
147. Moldovan GL, D'Andrea AD. How the Fanconi Anemia pathway guards the genome. *Annu Rev Genet*. 2009;43:223–49.
148. Conti MA, Saleh AD, Brinster LR, Cheng H, Chen Z, Cornelius S, et al. Conditional deletion of nonmuscle myosin II-A in mouse tongue epithelium results in squamous cell carcinoma. *Sci Rep*. 2015;5:14068.
149. Schramek D, Sendoel A, Segal JP, Beronja S, Heller E, Orianian D, et al. Direct in Vivo RNAi Screen Unveils Myosin IIa as a Tumor Suppressor of Squamous Cell Carcinomas. *Science*. 2014;343(6168):309–13.
150. Ma X, Adelstein RS. The role of vertebrate nonmuscle Myosin II in development and human disease. *Bioarchitecture*. 2014;4(3):88–102.
151. Bozic I, Reiter JG, Allen B, Antal T, Chatterjee K, Shah P, et al. Evolutionary dynamics of cancer in response to targeted combination therapy. *Elife*. 2013;2:e00747.
152. Mirghani H, Amen F, Blanchard P, Moreau F, Guigay J, Hartl DM, et al. Treatment de-escalation in HPV-positive oropharyngeal carcinoma: ongoing trials, critical issues and perspectives. *Int J Cancer*. 2015;136(7):1494–503.
153. Liu H, Cracchiolo JR, Beck TN, Serebriiskii IG, Golemis EA. EGFR inhibitors as therapeutic agents in head and neck cancer. In: Burtne B, Golemis EA, editors. *Molecular determinants of head and neck cancer*. 1st ed. New York: Springer New York; 2014. p. 55–90.
154. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med*. 2008;359(11):1116–27.
155. Burtne B, Goldwasser MA, Flood W, Mattar B, Forastiere AA, Eastern Cooperative Oncology G. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: an eastern cooperative oncology group study. *J Clin Oncol*. 2005;23(34):8646–54.
156. Harrington K, Berrier A, Robinson M, Remenar E, Housset M, de Mendoza FH, et al. Randomised Phase II study of oral lapatinib combined with chemoradiotherapy in patients with advanced squamous cell carcinoma of the head and neck: rationale for future randomised trials in human papilloma virus-negative disease. *Eur J Cancer*. 2013;49(7):1609–18.
157. Machiels JP, Haddad RI, Fayette J, Licitra LF, Tahara M, Vermorken JB, et al. Afatinib versus methotrexate as second-line treatment in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck progressing on or after platinum-based therapy (LUX-Head & Neck 1): an open-label, randomised phase 3 trial. *Lancet Oncol*. 2015;16(5):583–94.
158. Chong CR, Janne PA. The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat Med*. 2013;19(11):1389–400.
159. Dorsey K, Agulnik M. Promising new molecular targeted therapies in head and neck cancer. *Drugs*. 2013;73(4):315–25.
160. Suh Y, Amelio I, Guerrero Urbano T, Tavassoli M. Clinical update on cancer: molecular oncology of head and neck cancer. *Cell Death Dis*. 2014;5:e1018.
161. Sacco AG, Cohen EE. Current treatment options for recurrent or metastatic head and neck squamous cell carcinoma. *J Clin Oncol*. 2015;33(29):3305–13.
162. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. *Nat Rev Drug Discov*. 2015;14(2):130–46.
163. Shaw AT, Hsu PP, Awad MM, Engelman JA. Tyrosine kinase gene rearrangements in epithelial malignancies. *Nat Rev Cancer*. 2013;13(11):772–87.
164. Huang M, Shen AJ, Ding J, Geng MY. Molecularly targeted cancer therapy: some lessons from the past decade. *Trends Pharmacol Sci*. 2014;35(1):41–50.
165. Matzinger O, Viertel D, Tsoutsou P, Kadi L, Rigotti S, Zanna C, et al. The radiosensitizing activity of the SMAC-mimetic, Debio 1143, is TNF alpha-mediated in head and neck squamous cell carcinoma. *Radiother Oncol*. 2015;116(3):495–503.
166. Eytan DF, Snow GE, Carlson SG, Schiltz S, Chen Z, Van Waes C. Combination effects of SMAC mimetic birinapant with TNFalpha, TRAIL, and docetaxel in preclinical models of HNSCC. *Laryngoscope*. 2015;125(3):E118–24.
167. Mahoney KM, Rennert PD, Freeman GJ. Combination cancer immunotherapy and new immunomodulatory targets. *Nat Rev Drug Discov*. 2015;14(8):561–84.
168. Gettinger SN, Horn L, Gandhi L, Spigel DR, Antonia SJ, Rizvi NA, et al. Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol*. 2015;33(18):2004–U2032.
169. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373(17):1627–39.
170. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711–23.
171. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509–20.
172. Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *J Clin Oncol*. 2015;33(17):1974–82.
173. Kuss I, Hathaway B, Ferris RL, Gooding W, Whiteside TL. Decreased absolute counts of T lymphocyte subsets and their relation to disease in squamous cell carcinoma of the head and neck. *Clin Cancer Res*. 2004;10(11):3755–62.
174. Jie HB, Schuler PJ, Lee SC, Srivastava RM, Argiris A, Ferrone S, et al. CTLA-4(+) regulatory T cells increased in cetuximab-treated head and neck cancer

- patients suppress NK cell cytotoxicity and correlate with poor prognosis. *Cancer Res.* 2015;75(11):2200–10.
175. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer.* 2012;12(4):252–64.
 176. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med.* 2015;372(26):2521–32.
 177. Garon EB, Rizvi NA, Hui RN, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *New Engl J Med.* 2015;372(21):2018–28.
 178. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. *Lancet Oncol.* 2015;16(8):908–18.
 179. Sivan A, Corrales L, Hubert N, Williams JB, Aquino-Michaels K, Earley ZM, et al. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science.* 2015;350(6264):1084–9.
 180. Vetzou M, Pitt JM, Daillere R, Lepage P, Waldschmitt N, Flament C, et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science.* 2015;350(6264):1079–84.
 181. Weisenberger DJ. Characterizing DNA methylation alterations from the Cancer Genome Atlas. *J Clin Invest.* 2014;124(1):17–23.
 182. Jones PA, Baylin SB. The epigenomics of cancer. *Cell.* 2007;128(4):683–92.
 183. Lleras RA, Smith RV, Adrien LR, Schlecht NF, Burk RD, Harris TM, et al. Unique DNA methylation loci distinguish anatomic site and HPV status in head and neck squamous cell carcinoma. *Clin Cancer Res.* 2013;19(19):5444–55.
 184. Schlecht NF, Ben-Dayam M, Anayannis N, Lleras RA, Thomas C, Wang Y, et al. Epigenetic changes in the CDKN2A locus are associated with differential expression of P16INK4A and P14ARF in HPV-positive oropharyngeal squamous cell carcinoma. *Cancer Med.* 2015;4(3):342–53.
 185. Gaykalova DA, Vatapalli R, Wei Y, Tsai HL, Wang H, Zhang C, et al. Outlier analysis defines zinc finger gene family DNA methylation in tumors and saliva of head and neck cancer patients. *PLoS One.* 2015;10(11):e0142148.
 186. Timmermann S, Hinds PW, Munger K. Re-expression of endogenous p16(ink4a) in oral squamous cell carcinoma lines by 5-aza-2'-deoxycytidine treatment induces a senescence-like state. *Oncogene.* 1998;17(26):3445–53.
 187. Shi H, Chen X, Lu C, Gu CM, Jiang HW, Meng RW, Niu X, Huang YX, Lu MX. Association between P16(INK4a) promoter methylation and HNSCC: a meta-analysis of 21 published studies. *Plos One.* 2015;10(4):e0122302.
 188. Kostareli E, Holzinger D, Bogatyrova O, Hielscher T, Wichmann G, Keck M, et al. HPV-related methylation signature predicts survival in oropharyngeal squamous cell carcinomas. *J Clin Invest.* 2013;123(6):2488–501.
 189. Shi H, Chen X, Lu C, Gu C, Jiang H, Meng R, et al. Association between P16INK4a promoter methylation and HNSCC: a meta-analysis of 21 published studies. *PLoS One.* 2015;10(4):e0122302.
 190. Chen X, Liu L, Mims J, Punska EC, Williams KE, Zhao W, et al. Analysis of DNA methylation and gene expression in radiation-resistant head and neck tumors. *Epigenetics.* 2015;10(6):545–61.
 191. Le Tourneau C, Kamal M, Tsimberidou AM, Bedard P, Pierron G, Callens C, Rouleau E, Vincent-Salomon A, Servant N, Alt M, et al. Treatment algorithms based on tumor molecular profiling: the essence of precision medicine trials. *J Natl Cancer Inst.* 2016;108(4):d3v362.
 192. Le Tourneau C, Paoletti X, Servant N, Bieche I, Gentien D, Rio Frio T, et al. Randomised proof-of-concept phase II trial comparing targeted therapy based on tumour molecular profiling vs conventional therapy in patients with refractory cancer: results of the feasibility part of the SHIVA trial. *Br J Cancer.* 2014;111(1):17–24.
 193. Rodon J, Soria JC, Berger R, Batist G, Tsimberidou A, Bresson C, et al. Challenges in initiating and conducting personalized cancer therapy trials: perspectives from WINTHER, a Worldwide Innovative Network (WIN) Consortium trial. *Ann Oncol.* 2015;26(8):1791–8.
 194. Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res.* 2014;24(5):743–50.
 195. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus Docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med.* 2015;373(2):123–35.
 196. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med.* 2013;369(2):122–33.
 197. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science.* 2015;348(6230):124–8.
 198. Hariri S, Markowitz LE, Dunne EF, Unger ER. Population impact of HPV vaccines: summary of early evidence. *J Adolesc Health.* 2013;53(6):679–82.
 199. Baldur-Felskov B, Dehlendorff C, Munk C, Kjaer SK. Early impact of human papillomavirus vaccination on cervical neoplasia—nationwide follow-up of young Danish women. *J Natl Cancer Inst.* 2014;106(3):djt460.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

